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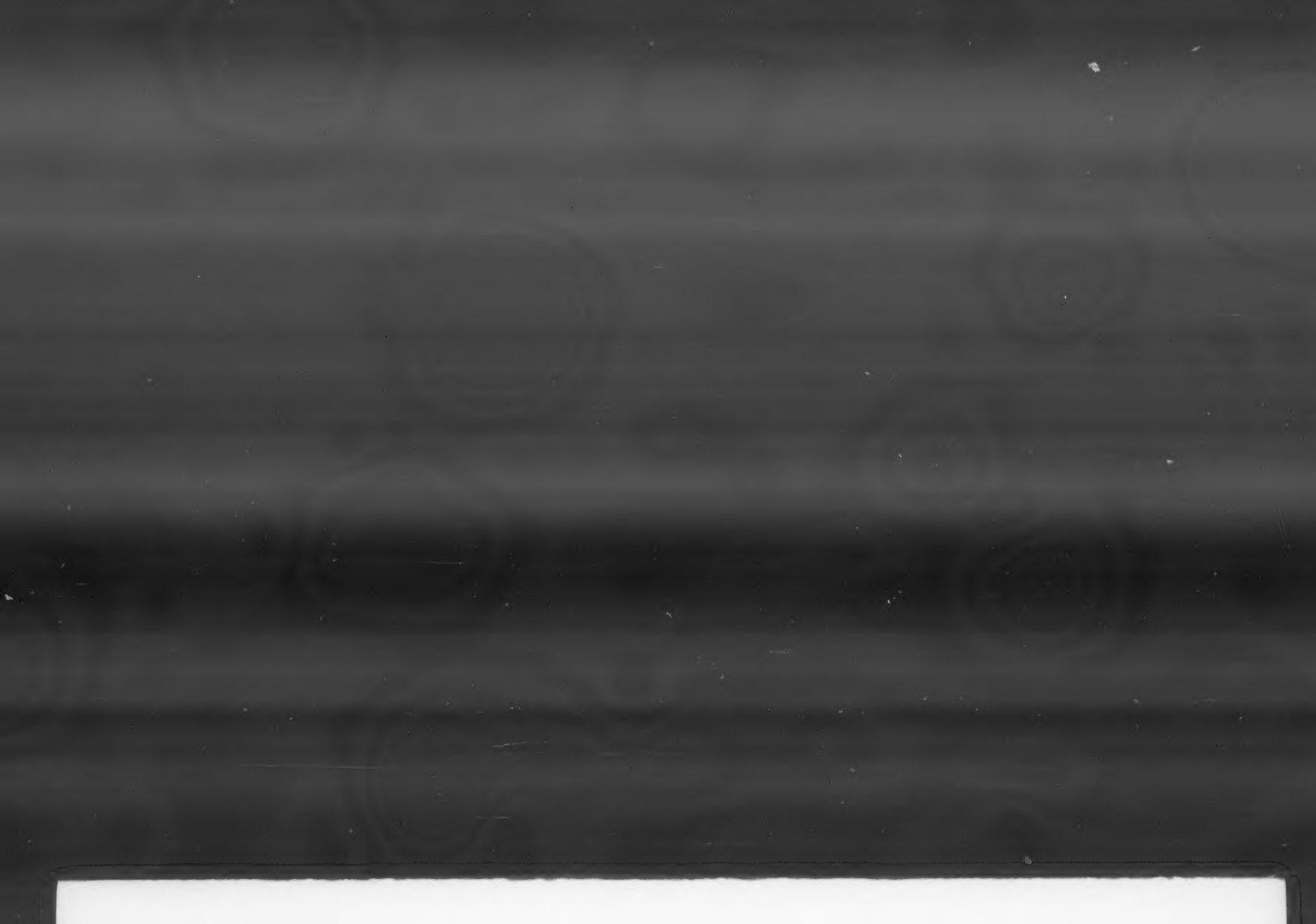

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Vol. 3 - Nº 1:

ANGIODIACTIN: A NEW VASODILATOR SUBSTANCE OF THE TISSUES, por *Juan Carlos Fasciolo.*

ON THE ABSORPTION MECHANISM OF PELLETS, por *J. Lewin and F. Huidobro.*

MECHANICAL RESPONSE OF SKELETAL MUSCLE IN NORMAL, HYPERTHYROID AND HYPORTHYROID RATS, por *F. Hoffman and B. Holmgren.*

THE INTERCHANGE OF CARBON DIOXIDE AND OXYGEN IN THE SKIN AND THE MUCOSAE OF HUMAN BEINGS, por *Alfredo Lanari.*

Nº 2 y 3:

ALEJANDRO LIPSCHUTZ, por *Bernardo A. Houssay.*

L'HIPERACTIVITÉ REACTIONNELLE DE L'OVAIRE RESTANT APRÈS HEMIOVARIECTOMIE, por *Max Aron et Claude Aron.*

THE ROLE OF OVARIAN SECRETIONS IN THE PATHOGENESIS OF CHEMICALLY INDUCED CANCERS, por *F. Bielschowsky.*

ALGUNAS REACCIONES HISTOQUIMICAS Y FUNCIONALES DE LOS MASTOCITOS, por *Washington Ruño.*

THE DISCOVERY OF SMOOTH MUSCLE IN THE UTERUS, por *George W. Corner.*

LA SÉRIE ALLÉNOLIQUE DES OESTROGÈNES ARTIFICIELS, por *R. Courrier.*

MECANISMOS QUE INFLUYEN EN EL EFECTO DIURÉTICO DE LA RENINA, por *H. Croxatto, L. Barnafi, M. de la Lastra, R. de la Parra y R. Vera.*

RELAÇOES GENÉTICAS ENTRE AS GÓNADAS E A GLANDULA CORTICO-SUPRARENAL, por *A. Celestino da Costa.*

EXTENDIDOS DEL SEDIMENTO URINARIO, por *E. B. del Castillo y J. Argonz.*

RELATION OF STILBOESTROL TO NATURALLY OCCURRING SUBSTANCES, por *E. C. Dodds.*

NEUROMUSCULAR ALTERATIONS PRODUCED BY MOTOR-NERVE SECTION, por *C. Eyzaguirre and J. V. Luco.*

FACTORS WHICH ACCELERATE OR DELAY THE APPARITION OF PANCREATIC DIABETES IN THE RAT, por *V. G. Foglia.*

SLOW TONIC WAVES IN THE TURTLE HEART (FANO PHENOMENON), por *B. Günther.*

ULTRAVIOLET ABSORPTION SPECTRA OF GENUINE OESTROGENS (INFLUENCE OF SOLVENT ON THE ABSORPTION SPECTRUM), por *Christian Hamburger.*

PROBLEMS OF REPRODUCTIVE HORMONE ACTION, por *John Hammond.*

INFLUENCE OF THYROID HORMONE ON THE HEART-VAGUS SYSTEM, por *F. Hoffmann, S. Middleton, A. Molina and J. Talesnik.*

ADRENAL TUMORS IN GONADECTOMIZED RATS, por *B. A. Houssay, A. B. Houssay, A. F. Cardeza, V. G. Foglia and R. M. Pinto.*

GLUTATHIONEMIA OF THE NORMAL RABBIT AND GUINEA-PIG, AND ITS VARIATIONS AFTER THE APPLICATION OF ALLOXAN, por *O. Koref, L. Vargas and A. Vukusic.*

INFLUENCE DES HORMONES SEXUELLES SUR LA PRODUCTION DU CANCER DU FOIE CHEZ LE RAT, por *A. Lacassagne, L. Hurst, F. Zajdela et R. Royer.*

INFLUENCE OF SULFASUXIDINE ON THE EFFECT OF ALPHA LIPOIC OR THIOCTIC ACID ON THE VOLUNTARY ALCOHOL INTAKE OF RATS DEPLETED OF FACTOR N_1, por *Jorge Mardones, Natividad Segovia, Arturo Hederra and Francisco Alcaíno.*

SOME ASPECTS OF THE MECHANISM OF BIOLOGICAL HYDROXILATION OF STEROIDS, por *K. Miescher, A. Wettstein and F. W. Kahnt.*

POSIBLE MECANISMO DE LA DEFIBRILACIÓN MIOCARDICA POR CONTRACHOQUE ELÉCTRICO, por *O. Orías.*

ON THE POSSIBLE SHARE OF ANDROGENS IN THE DEVELOPMENT OF SECONDARY SEX ORGANS IN THE FEMALE RAT, por *F. J. A. Paesi, E. M. van Soest and S. E. de Jongh.*

CONSERVATION OF GONADAL TISSUE FOR TRANSPLANTATION, por *A. S. Parkes.*

ADRENALS AND GONADOTROPHIN B, por *Christiane Dosne de Pasqualini.*

COMENTARIOS SOBRE EL USO TERAPÉUTICO DE ESTEROIDES PARAHORMONALES, por *Jaime Pi-Suñyer.*

CUATRO ETAPAS EN EL CONOCIMIENTO DEL SISTEMA NEUROVEGETATIVO, por *Augusto Pi-Suñer.*

THE EFFECTS OF PROGESTERONE AND RELATED COMPOUNDS ON OVULATION AND EARLY DEVELOPMENT IN THE RABBIT, por *Gregory Pincus and M. C. Chang.*

CANCER RESEARCH AS VIEWED ALONG THE YEARS, por *Peyton Rous.*

THE "CRITICAL PERIOD" IN THE DEVELOPMENT OF INFLAMMATION, por *Hans Selye.*

ATMUNGSGRÖSSE UND BLUT / HIRN-SCHRANKE, por *Hans Winterstein.*

THE LAW OF FOLLICULAR CONSTANCY, por *S. Zuckerman.*

Nº 4:

THE RESPONSES OF AXONS TO MECHANICAL STIMULI, por *A. Rosenblueth, R. Alvarez Buylla and J. García Ramos.*

A STANDARDIZED METHOD FOR THE NEUROGENIC AND CARDIOGENIC PRODUCTION OF VENTRICULAR ARRHYTHMIAS IN THE RAT, por *M. R. Malinow, F. F. Batlle and B. Malamud.*

THE RELATIONSHIP OF THE GONADS AND ADRENALS TO THE GROWTH OF HAIR IN MICE AND RATS, por *Alberto B. Houssay.*

STUDIES ON THE RELATION OF CATHEPSINS TO TO PROTEIN SYNTHESIS. THE EFFECT OF HORMONES, *L. C. Junqueira and Hanna A. Rothschild.*

A HEART-LUNG PREPARATION IN THE RAT, por *M. R. Malinow, F. F. Batlle and M. Malamud.*

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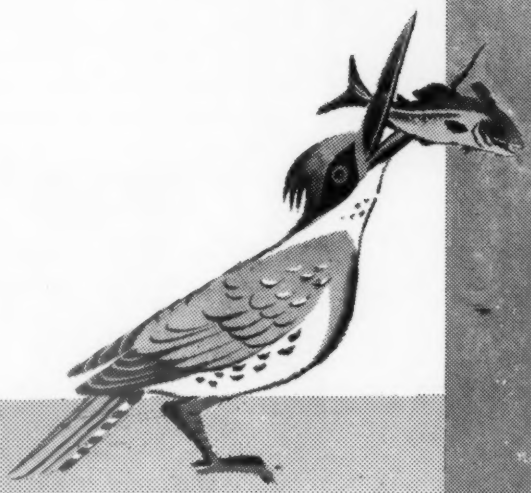

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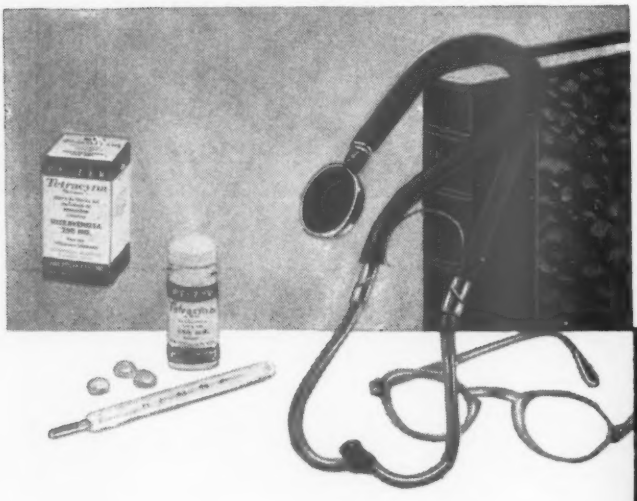

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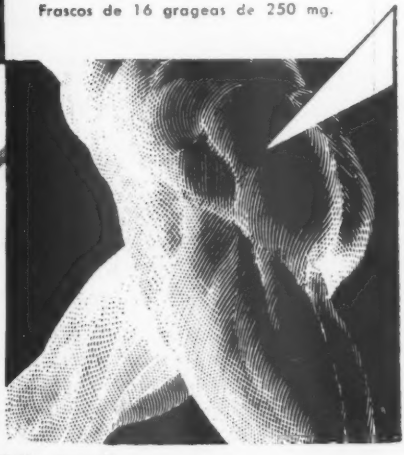

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A NOMOGRAPHIC SYSTEM FOR CALCULATION OF WATER REQUIREMENTS

GUILLERMO WHITTEMBURY M., MANUEL RAMÍREZ V., JAVIER FERNÁNDEZ Ñ. AND CARLOS MONGE C.

(Chair of Clinical Medicine, Medical School and Institute of Andean Biology. Lima, Perú. 1955)

IN NORMAL CONDITIONS the human body is in water balance or, in other words, the amount of water excreted is equal to the amount ingested. To appreciate the water demand, it is necessary to know the magnitude of the losses which depend on various factors. The tabular grouping, or in form of arithmetical Cartesian abacus of these factors, although being sufficiently descriptive, is not appropriate for an easy and exact reading; moreover, the greatest part of the graphs published include only some of the important factors in water balance, being then necessary to recur to two or more of them to obtain complete information.

With data obtained from the literature ([1], [2], [3]) a nomographic system of parallel logarithmic scales ([4]) has been constructed. The principal variables upon which the water balance depends in the human being are grouped.

Losses of water. — The water of the organism is lost by renal or extrarenal routes. The volume of urine must be sufficient for the elimination of the load offered to the kidney. This load is proportional to the caloric production or caloric ingestion. The urinary load is expressed in milliosmols per 24 hours, and depends on the quality of the diet: a) It is accepted that the ordinary diet produces more or less 1200 milliosmols per 24 hours; b) During fasting more or less 800 milliosmols are produced in 24 hours; mainly from the endogenous combustion of proteins; c) If protein catabolism of a fasting subject is blocked by administering 0.04 to 0.05 grams of carbohydrates per metabolized Calorie, the load is reduced to an approximate value of 600 milliosmols in 24 hours.

The concentration capacity of the kidney determines the urinary volume necessary to eliminate a given load offered to the kidney. For that reason the urinary volume depends on the concentration capacity, the load offered to the kidney and the water intake.

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The minimum urinary volume depends on the maximum concentration power of the kidney for a certain load. For that reason, the greater the concentration capacity of the kidney and the lower the load offered to it, the smaller will be the urinary volumen necessary for its elimination. That is why the minimum urinary volume fixed at 500 cm^3/24 h is arbitrary; for instance, the minimum urinary volume corresponding to an ordinary diet may be 850 cm^3/24 h while that of a subject on a hypercaloric, hypoproteinic and hyposaline diet may be of 150 cm^3/24 h if the kidney is in ideal conditions for maximum concentration.

The normal extrarenal losses are: a) insensible water loss (evaporation by skin and lungs); b) sweat; and c) faeces.

The insensible water loss is according to Darrow and Pratt of 0.42 cm^3/Cal/24 h. Newburgh ([3]), in an excellent revision of the literature, and with data of his own experience, accepts that 25 % of the total calories correspond to the insensible loss, that is to say 0.43 cm^3/Cal/24 h.

Although for most authors no sweat is produced in normal conditions, Darrow and Pratt accept a daily loss of 0.15 to 0.20 cm^3/Cal/24 h.

They say that the loss of water by the faeces is about 0.04 cm^3/Cal of diet in 24 hours. During fasting, stool water is negligible.

The nomographic system. — The construction of this nomographic system is based on values taken from Gamble ([2]) and Darrow and Pratt ([1]). We have taken as its basis an adult weighing 70 kilograms, healthy, in normal environmental conditions, with a production of 3000 Calories per 24 h and an insensible loss (skin and lungs) of 1260 cm^3 in 24 h. The loss by faeces and the increase in the insensible loss or sweat produced by a rise of temperature have not been taken into consideration. The nomographic scales have taken as fundamental variables: the urinary volume, the urinary osmolar concentration and the total osmolar excretion by the kidney; the evaporation by skin and lungs remains as a constant. Consequently in cases of abnormal losses, such as vomiting, diarrhea, profuse sweating, etc, the nomographic system maintains its value, but the calculation must be integrated with the values of these abnormal losses.

The nomographic system is composed of three fundamental scales (fig. 1), A-K-A'. The scale A is expressed in cm^3 per 24 hours and corresponds to urinary volumes (O) and total demand. The scale K is expressed in urinary milliosmols in 24 hours (total elimination of solutes). The scale A' is expressed in milliosmols per cubic centimeter of urine, and in the equivalent specific gravity. The scale B is equivalent to the scale A but expressed in cubic centimeters of water per kilogram of weight in 24 hours. The scale C is expressed in cubic centimeters of water per Calorie per 24 hours. The scales B' and C' are repetitions of A' to complete the nomographic system. The *a*, *b*, *c* and *d* segments of the scale K correspond to diverse osmolarities dependent on the diet. The segment *a* corresponds to an ordinary diet, *b* to a fasting subject, *c* to a fasting subject receiving 0.04 to 0.05 grams of glucose for each metabolized Calorie, and *d* to the administration of a hypercaloric, hypoproteinic and hyposaline diet.

The formulas employed in the construction of the nomographic system are: $K = Ao \times A'$ mosM/cm^3; where K is the total elimination of

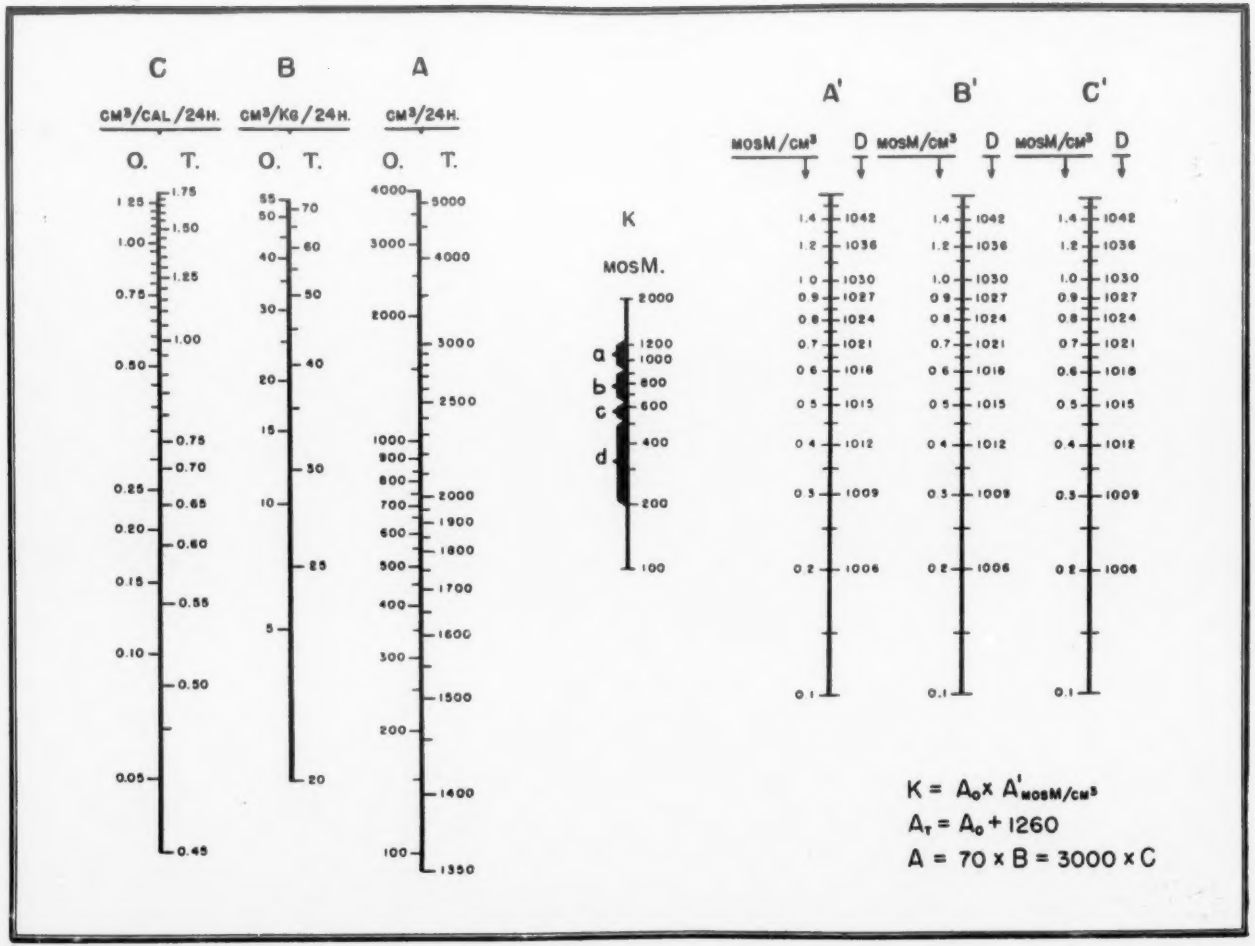

FIG. 1. — A', B', C': *urinary osmolar concentration (mosM/cm³) and corresponding urinary specific gravity (D).* K: *total excretion of solutes in urine of 24 hours, expressed in milliosmols (mosM);* a *ordinary diet;* b *fasting;* c *0.04 to 0.05 grams of glucose for each metabolized Calorie (fasting subject);* d *hypercaloric, hypoproteinic and hyposaline diet.* A: *urine volume (O) and total demand of water (T) in 24 hours (cm³/24 h) corresponding to a subject of 70 kg that metabolized 3000 Calories.* B: *the same values of A expressed per kilogram of weight (cm³/kg/24 h).* C: *the same values of A expressed per metabolized Calorie (cm³/Cal/24 h).*

solutes in urine in mosM/24 h; Ao is the urinary volume in cm^3/24 h, and A' mosM/cm^3 is the urinary osmolar concentration in mosM/cm^3. $A_T = Ao + 1260$; where A_T is the total demand of water in cm^3/24 h and 1260 is the insensible water loss. The scale *B* has been constructed with the values of *A* divided by 70 (kg). The scale *C* with the values of *A* divided by 3000 (Cal).

In the reading of the nomographic system the scales A-K-A'; or B-K-B'; or C-K-C' will be used, depending on the information needed. The reader must remember that the nomogram A-K-A' is constructed on the basis of 3000 Calories metabolized and 70 kg of body weight, therefore, it gives practically the maximum values for urine and total demand of water. The nomogram B-K-B', is based as the former in 3000 Calories, has the advantage of expressing the data corrected for kilograms of body weight, and therefore permits the calculation of the urinary and total water demands in subjects of any weight. The nomogram C-K-C' is undoubtedly the most useful because it considers the Calories metabolized by the subject, and in it all the described variables of the present work are considered.

The readings are made in the ordinary form, using a straight line uniting the desired values in each one of the three groups of scales. *Example 1.* - We want to know what will be the urinary volume and the total demand of water of a subject that eliminates in the urine of 24 hours 1200 milliosmols (ordinary diet) and for which a urinary specific gravity of 1.018 is desired. We take the value 1.018 in A' and unite it with a straight line with the 1200 in scale K; where the prolongation of this line cuts the scale A, gives us the answer: 2000 cm^3 of urinary volume (scale O) and 3300 cm^3 of total water demand (scale T). *Example 2.* - What is the amount of water needed by the patient ingesting a hypo-proteinic, hyposaline diet of 1800 calories, and eliminating urine with a specific gravity of 1.006? The scales C, K and C' will be used. Uniting 1.006 in scale C' with 400 mosM in K (this number is arbitrarily selected, according to what has already been mentioned), the prolongation of the straight line will cut C in 1.08 cm^3/Cal/24 h (scale T). As the caloric value of the diet is 1800 calories, multiplying $1.08 \times 1800 = 1944$ cm^3/24 h. The patient must receive approximately 1950 cm^3 of water in 24 hours. It is obvious that the nomogram may be used in any way, as long as two related variables are known.

Discussion

The importance of water balance in clinical medicine is universally accepted. Frequently the physician is contented with the knowledge of the absolute values for urinary volume, insensible loss and total demand of water. The nomographic system presented, gives at a glance an idea of the ample variations these values have, depending on body weight, diet, and urinary specific gravity. Darrow and Pratt (1) recommend in the calculation of total water expenditure to take as a basis a urinary specific gravity of 1.012, because by doing so, the kidney is left with a sufficient reserve to cover the small deficiencies that may occur due to losses by the faeces, by increase in the insensible perspiration and sweat.

The nomographic system not only has a practical value for the determination of the total water demand, but also serves to calculate the minimum urinary volumes corresponding to different diets and urinary specific gravities. Knowing the urinary specific gravity and volume of urine of a given patient, the physician may calculate the osmolar load eliminated in 24 hours and in this way know if the patient is eliminating or retaining solids. The nomographic system may also be applied to calculate the water demand in children, remembering only that the renal load of an artificially fed baby, falls in the lower part of the *a* zone of scale K, and if breast fed, falls nearly as low as the *c* zone of the scale K.

The values used for the construction of the nomographic system are necessarily only approximate, and must not, by any means, be taken with absolute rigidity. The intention of the authors has been to help the physician in the quantitative interpretation of water balance and its better application to individual cases, but they do not intend to replace the clinical judgement that must guide all balance studies in its practical applications.

Summary

A nomographic system is presented in order to calculate the urinary and total body requirements of water in ml/24 h; ml/kg/24 h and ml Cal 24 h. The fundamental variables used to construct the system were: total urinary solutes (mosM) in 24 hours, urinary osmolar concentrations (mosM/ml) and 24 hours urinary output. The insensible water loss was considered constant at 0.42 ml Cal/24 h, in the construction of the nomographic system. The practical applications of the nomographic system in clinical medicine are discussed.

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CONDITIONING OF TACHYCARDIA PRODUCED BY HISTOTOXIC ANOXIA

MAURICIO RUSSEK

(Neurophysiological Laboratories of the National School of Biological Sciences. México)

THE PRESENT STUDY has as a methodological basis, the concept of "functional system" [Anoghin (7)] according to which the circulatory and respiratory systems belong to a single functional unit, integrated at different levels of the nervous system, including the cortical level; this functional unit has been nominated "cardiorespiratory functional system", and its study started with a series of works about its afferent component [Alvarez-Buylla (1, 3, 5) and others].

Few data are available about the participation of the cerebral cortex on the integration of this system: in 1951, Alvarez-Buylla and Russek (6) presented a work describing the conditioning of the cardiorespiratory reactions produced by histotoxic anoxia; a year later, Viatkevitch (15) confirmed the conditioning of the respiratory reaction using anoxemic anoxia as the stimulus, and registering the blood saturation of oxygen; after that Belaus and Grebienkina (8) conditioned the tachypnoea produced by lobelin, using pneumographic recording.

This work was done in order to analize more closely the cardiac conditioned reflex obtained by the association of an exteroreceptor stimulus with the reactions produced by the injection of cyanide, with the aim of studying the participation of the cerebral cortex in the cardiorespiratory reactions produced by histotoxic anoxia.

METHODS

All the experiments were performed in a chamber of conditioned reflexes belonging to our laboratories; this chamber has been fully described by its author (2).

Ten experiments were realized in 7 different dogs, whose weight varied between 18 and 24 kg, in order to study the reactions produced by cyanide in dogs isolated in the chamber. In a male dog weighing 25.5 kg named "Golondrino", 113 experiments were made in which were

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obtained: the conditioning to tactile stimulus of tachycardia produced by cyanide, its extinction, reconditioning, differentiation and the production of an experimental neurosis while trying a difficult differentiation. In another male dog of 22 kg, named "Tiger", 14 experiments were realized during which the cardiac reaction produced by cyanide was conditioned to a luminous stimulus.

Cyanide was injected in the peritoneal cavity, by a system of remote injection already described (2), similar to Teitelbaum's and Gantt's (13).

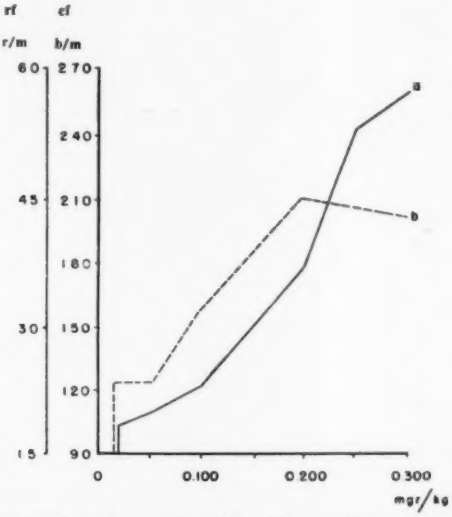

FIG. 1. — *Dog, conditioned reflexes chamber. Dose/effect curve of maximal tachycardia and tachypnoea produced by intraperitoneal injections of cyanide. Abscissas: dose of cyanide in milligrams per kilogram of bodyweight (mg/kg); ordinates: respiratory frequency (rf) in respirations per minute (r/m) and cardiac frequency (cf) in beats per minute (b/m). Full line: tachycardia; broken line: tachypnoea.*

To record the cardiac reactions, an electrocardiograph was used, which directly recorded on kymograph as already described (5). To record the respiratory reactions an oilskin pneumograph was used connected to a Marey's capsule.

RESULTS

The study of the participation of the cerebral cortex in the cardiorespiratory reactions produced by histotoxic anoxia, using the method of conditioned reflexes, requires the knowledge of the unconditioned reactions produced by cyanide.

a) *Cardiorespiratory reactions produced by cyanide in unanesthetized dogs.* — In 5 experiments made in 5 different dogs, the intraperitoneal injection of 0.02 to 0.6 mg/kg (milligrams per kilogram of bodyweight) of cyanide solution caused an increase in the cardiac and res-

piratory frequencies. The amplitude of respiratory movements increased, but could not be appreciated quantitatively by the pneumographic record.

Figure 1 shows a dose/effect curve of maximal values of tachycardia and tachypnoea produced by different doses of cyanide.

Based upon these results, for the production of unconditioned tachycardia, the dose of 0.2 mg/kg of cyanide by intraperitoneal route was selected, which produced an average raise in cardiac frequency to 180

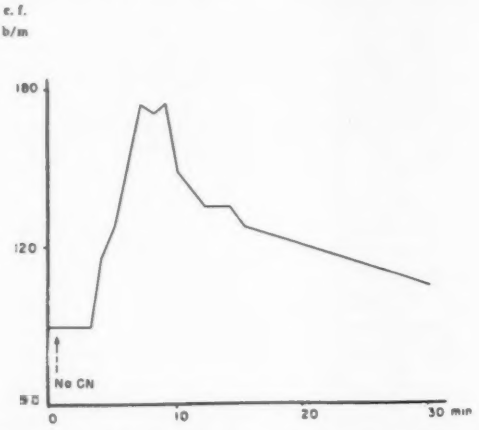

FIG. 2. — *Dog, conditioned reflexes chamber. Temporal developments of the tachycardic effect produced by the intraperitoneal injection of 0.2 mg/kg of an isotonic solution of sodium cyanide. Abscissas: time in minutes; ordinates: cardiac frequency in beats per minute. The arrow indicates the moment of injection.*

beats per minute (p/m) (230 % of the basal) (fig. 2) with an average latent period of 80 sec (extreme values: 60 to 120 sec.).

b) *Conditioning to a tactile stimulus of the tachycardia produced by the injection of 0.2 mg/kg of cyanide.* — The tactile stimulus (conditioned stimulus) in its first application, produced a slight tachycardia 20 sec afterwards, and immediately reached a maximum of 115 %, decreasing rapidly. This effect was caused by the "investigation reflex" ([11], [12]) that every new stimulus produces, and disappears after some repetitions.

To obtain the conditioning, the tactile stimulus was applied in the superior internal face of the front left leg, starting 3 sec before injecting the cyanide solution and continuing until the appearence of the tachycardia; in other words, the duration of the tactile stimulus was 100 sec, the cardiac reaction appearing in the last few seconds, equivalent to a conditioned reflex of "great delay" ([11], [12]).

About 2 to 5 sec after the injection, a tachycardia always appeared with an average maximum of 95 b/m (87-140) (120 %) and a duration of 15 sec (10-20), provoked by the introduction into the peritoneal

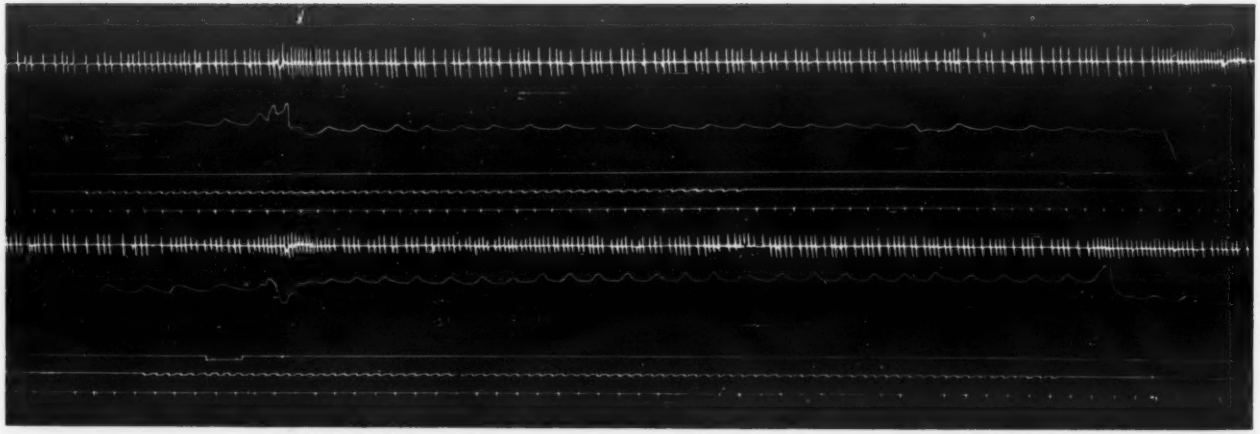

FIG. 3. — *Dog "Golondrino", conditioned reflexes chamber. Kymographic recording of the effects of the conditioned tactile stimulus without cyanide (upper record); and of the same stimulus accompanied of the injection of 0.2 mg/kg of cyanide (lower record). The tracings are in a descendent order: electrocardiogram, pneumogram, application of the injection of cyanide (in the lower record), application of the intermitent tactile stimulus and time (2 sec).*

cavity of the cyanide solution which in these experiments was not isotonic.

After 16 coincidences of the tactile stimulus with the cyanide injection (reinforcements), the former without cyanide, reproduced the initial tachycardia and the beginning of the tachycardia produced by histotoxic anoxia. In the kymographic records of fig. 3 and the temporal developments of fig. 4, the similarity between the effects of tactile stimulus applied alone, and together with cyanide can be appreciated; the first phase of tachycardia produced by tactile stimulus alone, which started after 5 sec and reached 120 b/m (170 %) corresponds to

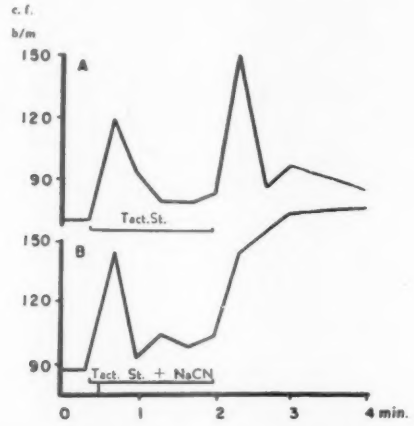

FIG. 4. — *Dog "Golondrino", conditioned reflexes chamber. Temporal development of the electrocardiographic records of fig. 3 A) Application of the conditioned tactile stimulus without the injection of cyanide. B) Application of the tactile stimulus accompanied by the injection of 0.2 mg/kg cyanide (arrow). Abscissas: time in minutes; ordinates: cardiac frequency in beats per minute.*

the conditioning of tachycardia produced by the introduction of the solution into the peritoneal cavity; the second phase, which started after 100 sec reached immediately a maximum of 150 b/m (215 %) and took 160 sec to completely disappear, corresponds to the conditioning of tachycardia produced by cyanide.

The respiratory reaction was also conditioned, as seen in fig. 3, but it was not analized, because the pneumographic record gave no quantitative data about the principal component in this reaction, that is: the increase in the respiratory volume.

Thirty reinforcements more were made, with the object of having a more steady conditioning; the experiments were then interrupted during 7 months in this dog, at the end of which the persistency of conditioning was again tested.

c) *Proof of persistency of the cardiac conditioned reflex.* — After 7 months of not introducing the dog in the chamber, the tactile stimulus applied without cyanide, produced the two phases of tachycardia already described, with the same magnitude and temporal relations as before

the period of rest. This means that the cardiac conditioned reflex presents great persistence to disappear when not being reinforced.

The tactile stimulus without cyanide was repeatedly applied in order to obtain the disappearence of the cardiac conditional reflex; it had to be repeated 75 times (in 50 days) to extinguish it. As "extinction criterion" the lack of effect after ten consecutive applications of tactile stimulus was adopted. This proves that the cardiac conditioned reflex presents also a great resistence to extinction by application of the conditional stimulus without the direct stimulus.

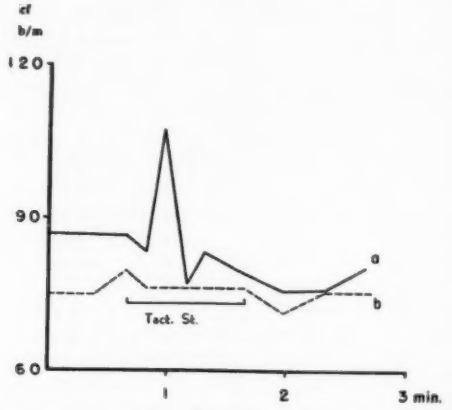

FIG. 5. — *Dog "Golondrino", conditioned reflexes chamber. Temporal development of the effects produced by the application of a conditioned tactile stimulus without cyanide (full line) and of the differentiated tactile stimulus (broken line). (Both records were obtained the same day). Abscissas: time in minutes; ordinates: cardiac frequency in beats per minute.*

d) *Reconditioning.* — After the extinction the cardiac conditioned reflex was again formed, but modifying the temporal relation between the conditioned stimulus and the cardiac response; the tactile stimulus was now started 1 sec after the injection, e. g. 20 to 40 sec before the beginning of tachycardia. In these experiments, the use of an isotonic cyanide solution was started, eliminating thus the tachycardic component immediate to injection.

After 8 reinforcements, the application of the tactile stimulus without cyanide produced after 40 sec a rise in the cardiac frequency of 28 b/m (35 %); after 19 reinforcements, the same stimulus produced after 20 sec a rise of 45 b/m (80 %).

Once the reconditioning obtained, the problem of establishing a differentiation was tackled.

e) *Differentiation between a point in the front left leg (conditioned point) and a point in the right hind leg.* — The delay of the reflex was diminished in order to facilitate the differentiation.

The effect of tactile stimulus without cyanide, applied to the left front leg and right hind leg was tried; in both points tachycardia was

produced; the application of the tactile stimulus without cyanide to the right hind leg was continued, alternating with the same stimulus with cyanide to the left front leg; after the fifth application to the right hind leg, no more tachycardia appeared when applied to that leg, but appeared when applied to the left front leg, where it had always been applied with cyanide. Fig. 5 shows that the tactile stimulus produced a tachycardia of 24 b/m (30 %) when applied to the left front leg, but did not produce any significant change in heart rate when applied to the right hind leg. (The record corresponds to the eighth application.)

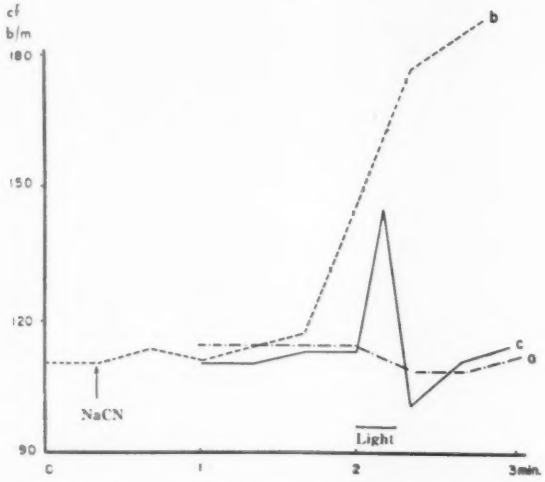

FIG. 6. — *Dog "Tiger", conditioned reflexes chamber. Conditioning of tachycardia produced by cyanide to a luminous stimulus; dots and dashes: effect of the luminous stimulus alone, before conditioning; broken line: effect of the injection of cyanide (0.2 mg/kg intraperitoneal) accompanied by tachycardia; full line: effect of luminous stimulus alone, after 20 reinforcements with cyanide. Abscissas: time in minutes; ordinates: cardiac frequency in beats per minute.*

f) *Obtention of an experimental neurosis when trying to differentiate two symmetrical points.* — After 30 applications of the tactile stimulus with cyanide to the left front leg, alternating with an equal number of applications of the same stimulus without cyanide to the symmetrical point of the right front leg, the conditioned effect disappeared not only from the point that was tried to differentiate, but also from the point that was always reinforced; the tactile stimulus alone, did not produce tachycardia when applied to the right leg, where it had never been accompanied with cyanide, nor when applied to the left leg where it had always been reinforced; sometimes it not only did not produce tachycardia but produced a slight bradycardia.

The disappearance of the conditioned effects, with inversion of the effect, together with the modification of the general behavior of the animal (it lost weight; it started to resist coming out of the cage and

enter the chamber; it stopped jumping spontaneously to the table; it shrieked and sometimes tried to bite; things it had never done before) indicated that an experimental neurosis was starting.

g) *Conditioning of the action of cyanide to a luminous stimulus.* — Once the conditioning of tachycardia produced by cyanide using a tactile stimulus as "signal" was studied, it was decided to try another stimulus (luminous) applying it during the rising phase of tachycardia and prolonging it during only 15 sec.

The luminous stimulus before conditioning did not produce any appreciable modification of cardiac frequency, as can be seen in fig. 6, the injection of cyanide producing the effect shown in curve "b" of the same figure; the luminous stimulus was applied seconds after the start of tachycardia produced by cyanide.

After 10 reinforcements the luminous stimulus without cyanide produced after 10 sec a rise in cardiac frequency of 30 b/m (38 %) and after 20 reinforcements the same effect, but with a latency of 4 sec (fig. 6, c).

Summarizing: an indifferent stimulus for the dog (tactile, or luminous), after coinciding a certain number of times with the injection of cyanide, acquired the property of reproducing the cardiac and respiratory initial effects of cyanide.

The cardiac conditioned reflex of tachycardia produced by cyanide was relatively easy to form, in spite of being of "great delay"; once formed, it presented great resistence to extinction; the differentiation of a distant point was easily obtained; the intent to differentiate two symmetrical points produced a neurosis.

Discussion

The results obtained in the present study clearly show, that a stimulus, indifferent at the beginning, acquires the property of producing tachycardia, up to 215 %, and hyperpnoea, after coinciding a certain number of times with the action of histotoxic anoxia; in other words, that the signals originated in the chemoreceptors as a consequence of an oxygen defficiency reach the cortex, where they can associate with any other stimulus that coincides in time with them; by this mechanism (formation of "temporal connections" [11, 12]) cardiac and respiratory conditioned reflexes of the responses to anoxia are established.

The analysis of the cardiac conditioned reflex gives some interesting data.

Buikow [9] affirmed that in order to form conditioned reflexes of the reactions originated in the interoceptors, a great number of reinforcements was required, in comparison to those necessary to form conditioned reflexes of reactions originated in exteroceptors; Belaus and Grebienkina [8] claim to have confirmed this, regarding to conditioning of the respiratory reactions originated in chemoreceptors (pneumographically recorded); they needed 40 to 60 reinforcements to form a conditioned reflex of "short delay" (5 to 10 sec) to the tachypnoea produced by lobelin.

The results of the present work show, that the tachycardia produced by cyanide was conditioned with 16 reinforcements, when the conditioned reflex was of "great delay" (100 sec), and with 10 reinforce-

ments when it was with "short delay". The establishment of the cardiac conditioned reflex of "great delay", in a relatively short number of reinforcements, indicates that the conditioning of this interoceptor reflex is easier to obtain than the conditioning of the salivary reaction initiated in exteroceptors, because Pavlov (11) reached the conclusion that it is not possible to establish a salivary conditioned reflex of "great delay" (more than 60 sec) if the simultaneous reflex is not previously formed.

The great persistency of the conditioning of tachycardia produced by cyanide, is similar to the one that accompanies the salivary and motor reflexes, observed by Gantt and Traugott (10).

The attainement of an experimental neurosis while trying to differentiate two symmetrical points with regard to conditioned tachycardia is interesting, because it demonstrates that the nervous conflicts based in neurovegetative reflexes originated in interoceptors are able of totally altering the cortical function, which is revealed in the modification of the general behaviour of the dog to the stimuli of the external medium. (The incapacity to differentiate symmetrical points has been described by Buikov [in Pavlov (10)] for salivary conditioned reflexes).

It may thus be concluded, that the cerebral cortex receives information of the chemosensitive interoceptors, permitting it to interfere in the regulation of the homeostatic reactions to oxygen defficiency in the same way that it receives information of other interoceptors and participates in the control of other internal processes as Buikov and collaborators have demonstrated (9). All these facts have confirmed Pavlov's idea (13) that the cerebral cortex is also the superior integrator organ of the vegetative reactions.

Conclusions

1. — The cardiorespiratory reactions produced by histotoxic anoxia may be conditioned to any exteroceptor stimulus.

2. — The cardiac conditioned reflex of tachycardia produced by histotoxic anoxia may be extinguished and reconditioned.

3. — The differentiation of a distant point of the skin is easily obtainable, but the intent of differentiating symmetrical points leads the dog to neurosis.

4. — As conclusion, the cardiac conditioned reflex of tachycardia produced by cyanide presents the same characteristics as the classical salivary conditioned reflex.

Summary

In unanesthetized dogs isolated in a conditioned reflexes chamber, provided with a system of injection at distance, and of electrocardiographical and pneumographic recording, 135 experiments were made. Using the method of conditioned reflexes, a study was made of the participation of the cerebral cortex in the cardiorespiratory reactions produced by histotoxic anoxia (intraperitoneal injections of a solution of sodium cyanide, in the dose of 0.2 mg/kg).

It has been shown that any stimulus that coincides a sufficient number

of times (10 to 20) with the injection of cyanide, is capable of producing tachycardia and tachypnoea similar to those obtained by histotoxic anoxia. In other words, the cardiorespiratory reactions that cyanide produces were conditioned to a tactile stimulus in one of the dogs, and to a visual stimulus in another.

The conditioned reflex formed, presented great resistence to extinction, and was conditioned in a short number of reinforcements.

The differentiation of a point of the skin distant to the conditioned, was easily obtainable, but the intent to differentiate the symmetrical point produced in the dog the initiation of an experimental neurosis.

The results stated lead us to the conclusion that the cerebral cortex receives information of the chemoreceptors, by which it participates in the regulation of the homeostatic mechanisms which compensate oxygen defficiency.

The author thanks Dr. J. J. Izquierdo for the economical aid lent by means of the National Institute of Scientific Investigation, for the realization of this work.

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SPASTIC ACTION OF THE VENOM OF THE SPIDER *PHONEUTRIA FERA*

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FROM THE CLASSIC publications of Vital Brazil and Vellard ([17]) refering to the toxic action of spider venoms, it is known that the poison of *Ctenus nigriventer* and *C. ferus* (= *Phoneutria fera* *) acts on the nervous and muscular system, and causes intense neurovegetative disturbances.

We have studied the muscular effects of this venom in order to localize its site of action.

METHODS AND MATERIAL

The venom of *Phoneutria fera* was used. It was obtained by electric excitation on these spiders **, according to the procedure described by us ([5]) with the addition of a glass plate perpendicular to the axis of the chelicera; the venom extractions were made every 8 to 10 days. To accomplish the comparative study, suspensions of triturated glands in physiological serum were made.

Electrophoretic study. — The technique proposed by Drewes ([9]) was used employing Whatman filter paper Nº 1. Duration: 3 hours; tension: 700 V; intensity: 3.5 mA; Buffer: Veronal - Sodium Veronal and Sodium chloride (Sodium Veronal concentration: 0.02 M); pH: 5.3; venom diluted 10 % in the same buffer.

To study the pharmacodynamic effect of each fraction, we made the elution of each of the paper stripes with physiological solution according to the procedure previously used by us ([2]).

Action on mammal's sketetal muscle. — Rat's soleus-gastrocnemius muscle were used and its isotonic contractions were recorded on a kymograph according to the technique described previously ([4, 15, 16]). We used as anaesthesia an average of 250 mg of chloral hydrate by intraperitoneal route.

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* According to W. Bücherl ([3]) *Phoneutria nigriventer* is synonymous to *P. fera*.

** We are grateful to Defensa Hnos. S. R. L. banana importers, for providing the spiders for these experiments.

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The venom was injected sometimes by intraperitoneal and other times by intravenous route (jugular vein). We always utilized unipolar and maximum stimulation.

In the final periods, when symptoms of asphyxia appeared, we used artificial respiration by cannulating the trachea.

Action on the batrachian's rectus abdominis. — The rectus abdominis of the *Bufo arenarum* Hensel, was inmersed in Ringer's solution (Na Cl 0.65 g; K Cl 0.014 g; Ca Cl_2 0.012 g; Na H CO_3 0.02 g for 100 ml distilled water) at room temperature, in a 10 ml bath, with air passage.

The following substances were tested on the poisoned muscle to check its effects:

Potassium chloride	5 %
Magnesium sulphate	5 %
Calcium chloride	5 %
Barium chloride	5 %
N-metil-O-metil-bebeerina chloride (Institute Vital Brazil)	0.025 % to 0.006 %
Prostigmin sulphate	0.05 %
Atropine sulphate	0.2 %

Results

Electrophoretic study. — As can be observed in fig. 1 the electrophoretic development corresponding to the gland extract, shows 2 fractions; one which migrates towards the negative and another towards the positive pole (2). In the case of the venom, only one fraction (1) of negative mobility is to be seen.

Immediate effects on the intact animal. — Administration of small amounts of *Phoneutria fera* venom, by intraperitoneal or intravenous route, causes, immediately after the injection. twitches, fibrillations, and spasms of the skeletal muscles, and hair raising, lachrymation, sialorrhoea, etc. The total poison or its fraction I have similar neuromuscular effects but fraction II lacks this action.

These tonic and clonic convulsions are similar to those provoked by the scorpion venom [Houssay (10); Del Pozo *et al.* (7, 8, 9)] and by the crotalic venom tipe I [Barrio and Vital Brazil (4)]. In fact, blepharospasm, spastic flexion of the fore legs and rigid extension of the body, hind legs and tail are observed in the initial period.

Following the spasmodic period, comes the paralysing phase which carries the animal to death by asphyxia.

Action on mammal's skeletal muscle (Rat's soleus-gastrocnemius). — The myogram also shows effects similar to those of the crotalic poison type I, and to the scorpion venom (see fig. 2, a-b) corresponding to each of the two phases observed in the intact animal.

Isolated stimuli (one by second) applied on the sciatic nerve or directely on the muscles, cause normal contractions followed by a slow and incomplete relaxation. Figure 4 shows a rise of the base line; this

phenomenom is more evident when tetanizing frequencies (from 50 cycles and more) are used, because the muscle remains contracted and shows fibrillations long after the stimulus has stopped (fig. 5).

After this period, the spasmodic contractions give way to a phase of loss of excitability, indirect first, and direct aftewards. Prostigmin, at this moment, does not cause the reappearance of the muscular response to the indirect stimulation.

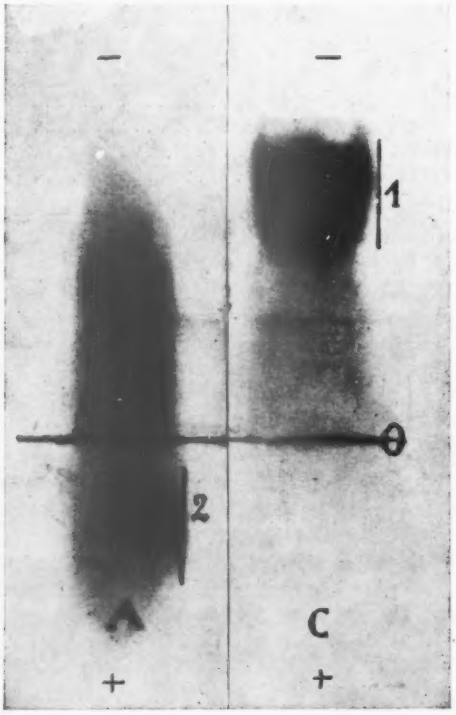

FIG. 1. — *Electrophoretic development on filter paper. A) Aqueous extract of complete gland of* Phoneutria fera. *C)* Phoneutria fera *venom obtained by excitation. 1, 2) Fractions isolated (see text).*

As the symptoms of asphyxia appear, before block occurs, it can be assumed that the former are due, in part, to the central depressing action of this venom.

It should be emphasized that the injection of glandular extracts * (up to 10 glands), in many occasions, did not provoke the iterative response, causing instead, the second phase, that is, the neuromuscular block. Prostigmin fails in this case to restore the excitability (fig. 3).

* Glands preserved in glycerine, kindly sent by Dr. W. Bücherl of the Butantan Institute, were used.

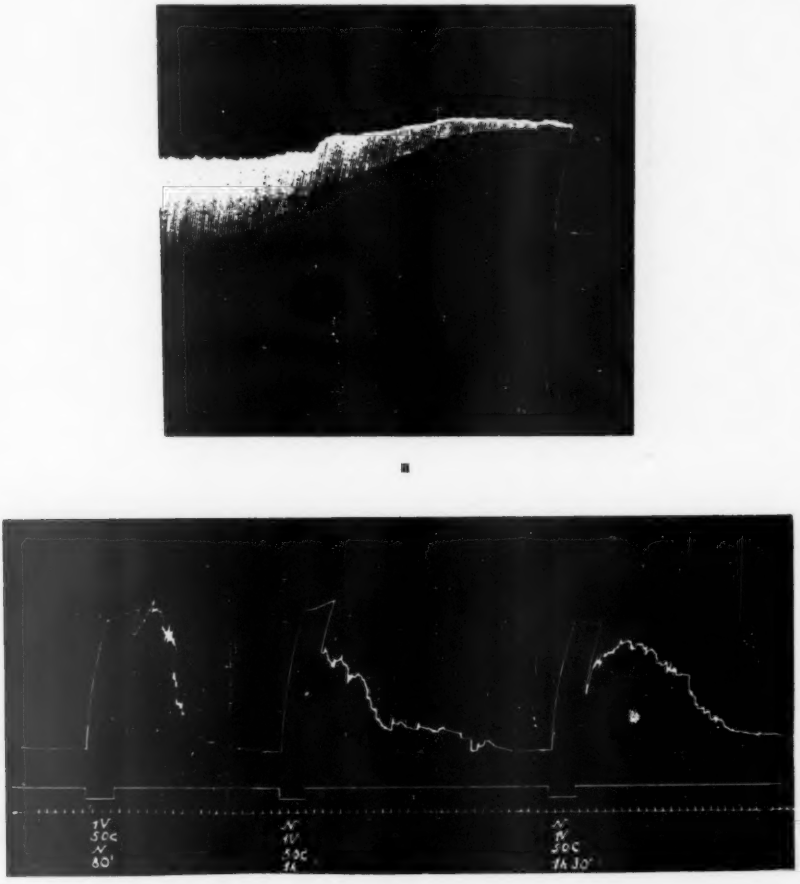

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FIG. 2. — Phoneutria fera *venom, effect on the rat's soleus-gastrocnemius.*
a) *30 Stimuli per minute on the sciatic, 15 minutes after the injection, of 0.5 mg. i. p. of venom. The rise of the base line caused by the deficient muscular relaxation may be observed.*
Another preparation: b) *50 Cycles per second, 80 minutes after the injection of 0.8 mg i. p. of venom; sciatic stimulation. Time 6 seconds.*
After the stimulation ceases, fibrillations appear and relaxation is slow.

The *Phoneutria fera* venom has no action on the nerve because, when put in contact 30 minutes with the sciatic, the repetitive response is not obtained with or without stimulation. The injection of the venom in the muscular mass or its superficial application on the innervated or recently denervated gastrocnemius provokes nearly immediately fibrillations and twitches. On the contrary, these phenomena are absent

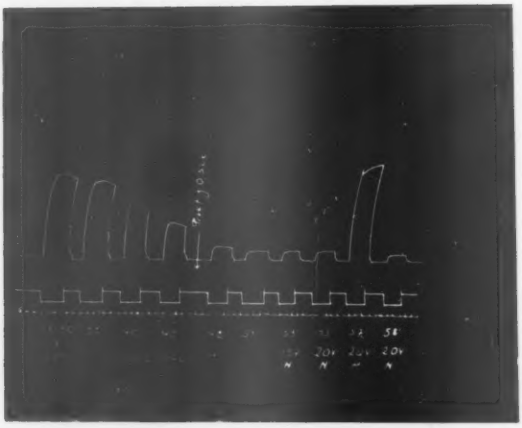

FIG. 3. — *Lack of repetitive response after the injection of 10 glands of* Phoneutria fera. *Voltage, frequency and site of stimulation is indicated under each contraction of the rat's soleus-gastrocnemius.*
After 2 hours and 30 minutes of the gland's extract injection, the slow relaxation or fibrillations are no more observed. At 2 hours and 45 minutes diminution of the contraction's amplitude (initiation of block). Injection of 0.5 ml of Prostigmin 0.05 % has no action against blockade. At 2 hours and 57 minutes the muscle responds with normal contractions, when directly stimulated, but does not respond when stimulated through the nerve.

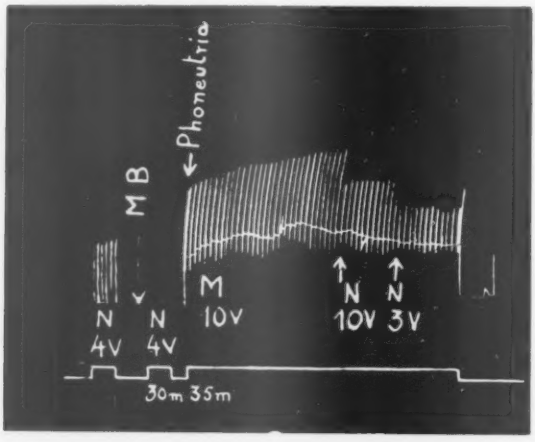

FIG. 4. — *Anticurare action of* Phoneutria fera *venom on rat's soleus-gastrocnemius. At the start: 30 stimuli of 4 volts por minute. Injection of 0.25 mg i. p. of metilbebeerina (curare). At 30 min complete block.*
Then 0.08 mg of Phoneutria fera *venom intravenously. Muscle (10 V) and nerve stimulation (10 and 3 V), provoke repetitive responses showing disappearance of the neuromuscular block.*

when the venom is injected in the muscle denervated 10 days before. This fact suggests that it has no action on the muscular tissue, or if any, not easily perceived.

The venom has decurarizing properties similar to those of veratrine ([12]), rattle snake tipe I * and scorpion poisons ([7]). As can be appreciated in fig. 4, after a complete block to the isolated impulses provoked by the curare (metilbebeerina), the responses to the nervous stimulation immediately reappear following the intravenous injection of the *Phoneutria fera* poison. The same occurs (fig. 6) when tetanizing

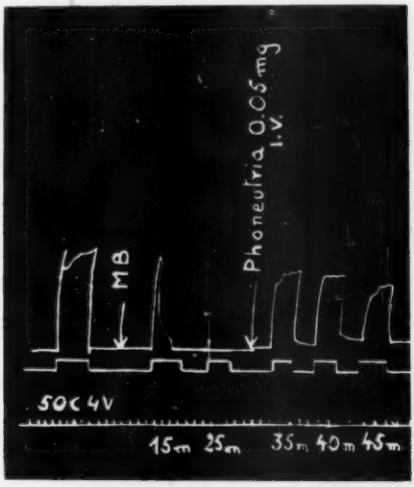

FIG. 5. — *Decurarizing effect of* Phoneutria fera *venom observed on rat's soleus-gastrocnemius.*
Stimulation of the sciatic with 50 cycles per seconds and 4 volts.
Injection of 0.25 mg of metilbebeerina (curare) i. p. At 15 minutes a marked Wedensky inhibition is observed. Ah 25 minutes the block is complete. Then injection in the jugular vein of Phoneutria fera *venom. At 35 minutes decurarization is complete, the muscle responds again to the indirect stimulation and the inhibition disappears.*
Time: 6 seconds.

stimuli are used; the injection of venom causes the disappearance of the Wedensky inhibition.

Action on the toad's rectus abdominis. — Bacq ([1]) has shown that veratrine and similar substances sensitize this muscle to the action of K and Ba ions.

Recently Moussatche and Vieira ([13]) clearly demonstrated that the crotalic poison type I has the same properties.

We have been able to prove that the total *Phoneutria fera* poison and the electrophoretic fraction I of negative mobility also sensitize the toad's rectus abdominis to K Cl and to Ba Cl. We should add that the same applies to all the amphibian's skeletal muscles tested.

* Studies made by us after our paper was published ([1]) showed that the crotalic venom type I has decurarizing action.

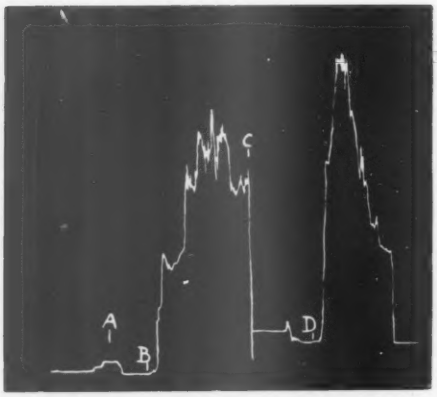

FIG. 6. — *Isolated rectus abdominis of* Bufo-arenarum *in a 10 ml bath.*
Stimulation each 3 to 5 minutes.
Action of Phoneutria fera *venom and potassium chloride.*
A — *Potassium chloride 5 % - 0.1 ml.*
B — *After washing:* Phoneutria fera *venom 0.05 % - 0.1 ml (direct action).*
C — *Washing (10 times).*
D — *Potassium chloride 5 % - 0.1 ml (potentation).*

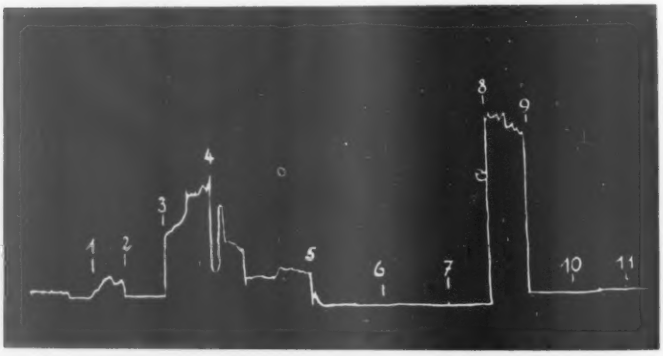

FIG. 7. — *Isolated* Bufo-arenarum *rectus abdominis in a 10 ml bath.*
Stimulation each 3 to 5 minutes.
Action of Phoneutria fera *venom and potassium chloride. Its antagonism with calcium chloride and magnesium sulphate.*
1 - *Potassium chloride 5 % - 0.05 ml.* 2 - *Washing.* 3 - Phoneutria fera *venom 0.01 % - 0.05 ml (slow contraction which appears at 3 minutes, then twitchings, even after washing it remains contracted) (direct action).* 4 - *Washing.* 5 - *Calcium chloride 5 % - 0.15 ml.* 6 - *Potassium chloride 5 % - 0.05 ml.* 7 - *Washing.* 8 - *Potassium chloride 5 % - 0.05 ml.* 9 - *Washing.* 10 - *Magnesium sulphate 5.% - 0.15 ml.* 11 - *Potassium chloride 5 % - 0.05 ml.*

The potentiating action of the venom on the effect of K Cl is shown in figs. 6 and 7. The same happens with Ba Cl_2 (fig. 8) which does not provoke a direct response before the muscle is sensitized; after sensitization, a sustained contraction is obtained.

As can be observed in fig. 6, this venom causes, in high dilutions (0.05 %), the contraction of the rectus abdominis, an effect which the

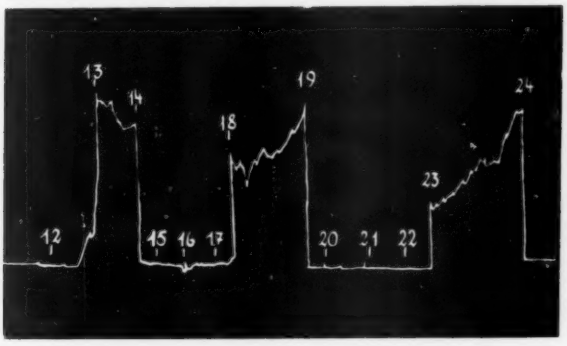

FIG. 8. — *The same as the previous preparation.*
Barium chloride's action and calcium chloride and magnesium sulphate antagonism. 12 - *Washing.* 13 - *Potassium chloride 5 % - 0.05 ml.* 14 - *Washing.* 15 - *Calcium chloride 5 % - 0.15 ml.* 16 - *Barium chloride 5 % - 0.05 ml.* 17 - *Washing.* 18 - *Barium chloride 5 % - 0.05 ml.* 19 - *Washing (the contraction yields very slowly falling rapidly if calcium chloride or magnesium sulphate are added).* 20 - *Magnesium sulphate 5 % - 0.15 ml.* 21 - *Barium chloride 5 % - 0.05 ml.* 22 - *Washing.* 23 - *Barium chloride 5 % - 0.05 ml. (there is still venom's activity).* 24 - *Washing - magnesium sulphate 5 % - 0.15 ml afterwards.*

scorpion venom has (*) but is not produced by the crotalic venom type I, even in great doses as we were able to prove.

The *Phoneutria* poison is strongly bound to the muscle (probably more on the motor end-plate) since its action persists even after many washings (fig. 6 and 7).

Figures 7 and 8 show the inhibiting action of magnesium sulphate and calcium chloride on the contractions produced by the *Phoneutria fera* venom and potassium and barium salts, the same as occurs with veratrine and crotalic poison type I. If the dose of venom is insufficient, the inhibiting action is absent (fig. 9).

Considering the antagonism of curare and *Phoneutria* venom, manifested by the decurarizing effect of the latter upon the soleus-gastrocnemius, we were also able to prove that curare impedes the toad's rectus adbominis contraction caused by the *Phoneutria venom* (fig. 10). After many washings, the sensitivity to potassium chloride appears, showing that the venom has more affinity for the muscle (motor end-plate) than curare.

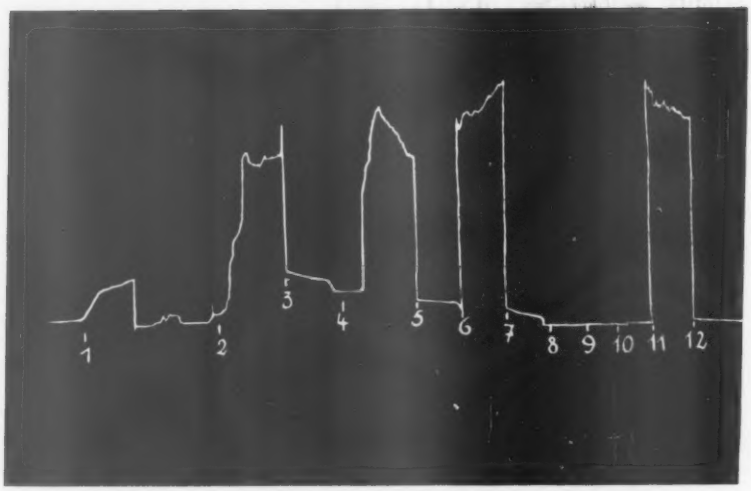

FIG. 9. — *Isolated* Bufo-arenarum *rectus abdominis in a 10 ml bath.*
Action of several doses of magnesium sulphate.
1 - *Potassium chloride 5 % - 0.1 ml.* 2 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 3 - *Magnesium sulphate 5 % - 0.1 ml.* 4 - *Plain washing. After the magnesium sulphate's effect is gone, the contraction reappears.* 5 - *Magnesium sulphate 5 % - 0.1 ml (insufficient dose).* 6 - *Potassium chloride 5 % - 0.3 ml.* 7 - *Washing.* 8 - *Magnesium sulphate 5 % - 0.3 ml (suficient dose).* 9 - *Potassium chloride 5 % - 0.1 ml.* 10 - *Washing.* 11 - *Potassium chloride 5 % - 0.1 ml.* 12 - *Washing.*

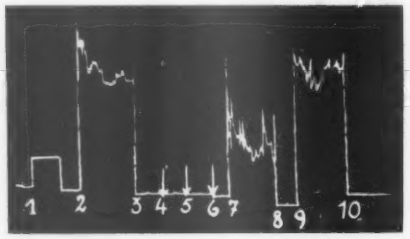

FIG. 10. — Bufo-arenarum *rectus abdominis in a 10 ml bath.*
Stimulation each 3 to 5 minutes.
Antagonism between curare (N-metil-O-metil-bebeerina) and the Phoneutria fera, *venom.*
1 - *Potassium chloride 5 % - 0.1 ml.* 2 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 3 - *N-metil-O-metil-bebeerina 0.006 % - 0.2 ml (15 minutes).* 4 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 5 - *Potassium chloride 5 % - 0.1 ml.* 6 - *Multiple washings.* 7 - *Potassium chloride 5 % - 0.1 ml.* 8 - *Washings.* 9 - *Potassium chloride 5 % - 0.1 ml.*

These facts strengthen the hypothesis that the muscular activation provoked by the venom is due fundamentally to its action at the neuromuscular junction.

Atropine (fig. 11) inhibits also the contraction provoked by the venom, alone or with the addition of K Cl, demonstrating that the venom has an acetylcholine-like activity.

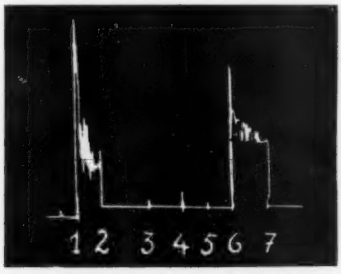

FIG. 11. — Bufo-arenarum *rectus abdominis in a 10 ml bath.*
Stimulation each 3 to 5 minutes.
Inhibiting effect of atropine on Phoneutria fera *venom produced contractions.*
1 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 2 - *Washing.* 3 - *Atropine sulphate 0.2 % - 0.2 ml.* 4 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 5 - *Washing.* 6 - Phoneutria fera *venom 0.05 % - 0.1 ml.* 7 - *Washing.*

DISCUSSION

The pharmacodynamic action of the *Phoneutria fera* venom on the skeletal muscle is manifested by spasms, fibrillations, spontaneous fasciculations and repetitive response *. This action is probably exercised at the level of the motor end-plate. We base this belief on the lack of action of the venom on the denervated muscle or on the nerve activity "in vivo" and "in vitro".

Though we have not studied it, this venom probably has an anticholinesterasic action, since the venom and tissues' extracts of other arthopoda have it. Even so, its stimulating action on the muscle should not be attributed to this property because, as Del Pozo and Derbez (8) have demonstrated with scorpion venom, those muscular effects are not the result of the inhibition of cholinesterase action.

In the second phase (paralytic) of the venom's action, a blockade of the indirect stimulation appears, which does not yield to curare or Prostigmin. The mechanism of this action should be further studied. The paralyzing principle may be different from the activating one, because, in a few cases, neuromuscular block was obtained with glandular extracts in the absence of a previous phase of fibrillations and repetitive responses. But this would not explain the chronological order in which the two phases always appear when total venom is administered.

The study of its action on the toad's rectus abdominis show the

* Probably a contracture component is added to these muscular responses.

muscle's sensitization to potassium and barium chloride, as well as its antagonism with magnesium sulphate, calcium chloride, curare (metilbebeerina) and atropine.

Veratrine. *Crotalus terrificus terrificus* type I, *Phoneutria fera* and the scorpion venoms have in general, a similar neuromuscular action. For this reason, we believe it is justified to classify these animals' venoms as "veratrine-form", the same as other ophidians and arachnidians venoms are called "curare-form". We should make clear that similarity does not mean absolute identity.

Further investigations on the pharmacodynamic action of these substances are justified, as well as a comparative study of their neuromuscular activity, recording the electrical potentials produced.

Some of the principal resemblances and differences of these venoms known at present are the following.

Veratrine, the same as crotalic (type I), scorpion and *Phoneutria fera* venoms cause a poisoning which with large doses, evolutions in 2 successive phases:

a) Polyphasic contractions with slow relaxation (repetitive response).
b) Paralysis with neuromuscular block.

They all sensitize the batrachian's rectus abdominis to K and Ba, being antagonized by Ca and Mg.

On the other hand, the *Phoneutria fera* and scorpion venoms have a direct muscle stimulating action "in vitro", but crotalic venom type I, lacks this property.

A tendency to the propagation and generalization of the spasms and fibrillations is observed which may be due to K liberation (the scorpion venom would also activate the spinal motor neurons). In all cases the neuromuscular block is not checked by curare nor by Prostigmin.

Thus, all possess decurarizing properties but, "in vitro", the sensitizing action of the rattle snake venom is not checked by curare nor by atropine ([3]).

Veratrine ([12]) as well as the scorpion ([7]), the *Phoneutria* and possibly the *Crotalus terrificus*, type I venoms, also increase the responses to acetylcholine.

The scorpion venom has a remarkable action upon the central nervous system [Del Pozo ([7]), Odoriz ([18])], a property possessed also by the crotolic [Houssay ([11])] and the *Phoneutria fera* venoms.

On the other hand, while veratrine has a definite action upon the nerve fibres, this is too weak, or does not exist in the venoms. The same applies to its action upon the denervated muscle which has not been sufficiently studied.

We may add that the poisoning by the spider *Latrodectus mactans* reminds the one produced by the *Phoneutria fera*, specially in regard to the fibrillations and muscular contractions which it causes. Notwithstanding, these effects are exercised by *Latrodectus* venom through the central nervous system ([14]). Also, as we have proved ([3]), it lacks the stimulating action on the sciatic-soleus-gastrocnemius and does not

cause the contraction of the batrachian's rectus abdominis nor sensitizes it to K and Ba, even in great doses.

SUMMARY

The spasmodic action of the *Phoneutria fera* venom and of some of its fractions isolated by electrophoresis was studied.

The poisoning evolves in 2 phases: 1º) Fibrillations and muscular spasms. 2º) Paralysis and asphyxia. Each one of these periods corresponds to characteristic phases of the neuromuscular activity studied on the soleus-gastrocnemius, that is: 1º) polyphasic contractions and slow relaxation (repetitive responses); 2º) neuromuscular block.

This venom lacks action on the nerve fibre and on the denervated muscle, having otherwise remarkable decurarizing properties.

It acts directly on the batrachian rectus abdominis, provoking intense muscular fibrillations and twitches and sensitizes it to potassium chloride and barium chloride. Its effects "in vitro" are antagonized by calcium chloride, magnesium sulphate, atropine and curare.

All these observations show the peripheral origin of these phenomena, the action of the venom is exercised probably in the region of the motor end-plate.

In the last asphyctic period it is possible that the central depressing action of the venom be added to the other.

Finally the properties of veratrine, and the *Phoneutria fera, Crotalus terrificus terrificus* type I and scorpion venoms are compared.

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THE TUMOR SIZE AS A FACTOR INFLUENCING THE TRANSPLANTABILITY AND LETHALITY PROPERTIES OF MAMMARY TUMORS IN MICE

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THE SUCCESS or failure in the transplantation of mammary tumors depends primarily on the genetic relationship between the host and the tumor used for transplantation. In other words, the tumor and the host must be genetically related. Such concept has been widely accepted since the work of Little, Strong, Bittner and some others, as it has been reviewed by Bittner (1931, 1935); Little (1941); Gorer (1948) and Snell (1953).

There are, however, some other factors influencing in one way or another the fate of the transplant. Some of them are related to the conditions of the host (age, diet, sex, some endocrine disturbances, etc.), others related to the tumor itself (type of tumor, number of previous passages, etc.), and still some others related to the technic of transplantation (site of the inoculation, concentration, etc, etc.). (See review by Little, 1941).

While studying some aspects of the growth of spontaneous and transplanted mammary tumors submitted to various experimental conditions, it was noticed that the spontaneous as well as the transplanted tumors had a typical growth curve characterized by its sigmoidal shape. That is, that tumors growing in the same host showed different growth patterns characterized by an initial phase of slow growth, followed without limits by a rapid growth phase and ending with a slow phase again near the date of spontaneous death of the host.

Due to these findings it was tentatively thought that perhaps such progressive changes in the rate of growth might be an indication of changes in some other properties of tumors. Consequently, it was decided to test the transplantability and lethality properties of spontaneous, as well as of transplanted mammary tumors when inoculated at different sizes.

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Browning (1948), working with either heterologous or homologous transplantations made by trocar into the anterior chamber of the eye, showed that in spontaneous tumors in mice, "autonomy" was not present in small tumors but it appeared gradually with increasing age of the tumors. In our experiments "transplantability" is defined as the "ability to grow progressively in a homologous host", and "lethality" as the "ability to kill the host".

METHOD

Mammary tumors arising spontaneously in mice of the Z (C_3H) or A stocks, and tumors of the same stocks that have been maintained in this laboratory by successive transplantations in genetically related mice, have been used.

Young female and male mice of the ZBC and ABC strains were used as recipient hosts. The ZBC mice are hybrids made up to grow tumors from the Z (C_3H) strain, whereas mice of the ABC stock are made up to grow tumors from mice of the A strain. The transplantation of the tumors was made in the following way: Tumors were excised, divided in small pieces with scissors and then homogenized in a Potter-Elvejehm homogenizer, suspended in saline and injected at different concentrations, either subcutaneously or intravenously. When the tumor was inoculated subcutaneously, the implant was made in the right side of the body of the recipient mouse by using a 22 gauge needle attached to a 2 cc syringe. When the tumor was transplanted intravenously the inoculation was made into the tail vein by using a 27 gauge needle. The size of the inoculum as well as the tumor cell concentration varied according to the type of experiment performed.

The size of the tumors used for transplantation was determined by measuring at right angles the two largest diameters with calipers, and the mean size calculated arbitrarily by the formula "Mean Size" $= \sqrt{a \times b}$, a and b being the measured diameters.

Since the main purposes of these experiments were to determine transplantability as well as lethality, the "take time" of the tumor after subcutaneous inoculation and the "death time" of the hosts were determined. The "take time" was defined as the time in days required for 50 % of mice of the various groups to develop a tumor in the site of the inoculation with a mean size of no less than 0.8 cm. Similarly, the "death time" was defined as the time in days required for 50 % of the animals to succumb to the tumor (Shear *et al.*, 1951; Miroff, Martínez and Bittner, 1955; Martínez, Miroff and Bittner, 1955).

When the inoculation was made intravenously, groups of mice were sacrificed at different time intervals after the inoculation and the number of lung nodules counted on the surface of the lungs under dissecting scope (1.3×10 magnification). The tumorous nature of the nodules was determined by histological sections.

RESULTS

A. *Take time of a large and a small tumor arising spontaneously in the same mouse.* — In this experiment, the take time of two mammary

tumors arising spontaneously in an A female mouse was determined. One of the tumors located in position R_4 had an average size of 2.60 cm, and the other, located in position L_2, had a mean size of 1.25 cm. Both tumors were excised at the same time and a tumor cell suspension prepared from each one. Two groups of young female mice of the ABC stock were used as recipient hosts. One group was then inoculated with 0.25 cc of the suspension prepared from the large tumor at a 2.5 % concentration, and the second group with the same amount of the suspension taken from the small tumor at a 3.5 % concentration. Once every other day, mice of both groups were checked for the appearance of tumors.

TABLE I

Effect of tumor size on the take time after transplantation

Series	Experiment	Tumor	Size cm (Mean)	Tumor cell concentration %	Nº of mice with tumors	Take time (days)
A	124 K	A spontaneous	2.6	2.5	6/6	21
	124 K	A spontaneous	1.2	3.5	5/7	39
B	124 K_1	A transplanted	2.2	5.0	9/9	20
	124 K_2	A transplanted		5.0	6/6	21

The results are shown in Table I-A. It can be seen that the "take time" for the group inoculated with the large tumor was 21 days, while it was 39 days in the group inoculated with the small. Also, the number of tumors obtained in both groups was different, for the large tumor produced new tumors in all mice inoculated, while 2 out of 7 mice of the group inoculated with the small failed to develop tumors.

During the course of this experiment, it was noticed that one mouse from each preceding group developed a tumor of the same time and the tumors attained an average size of 2.20 and 2.21 cm, respectively, at the same time. The "take time" of each of those tumors was newly determined by inoculating them at the same concentration into two groups of normal ABC female mice. The results shown in Table I-B indicated that the "take time" in both groups was now almost identical (20 and 21 days, respectively).

A similar experiment, using two equal size transplanted Z (C_3H) tumors, gave comparable results.

B. *Time of transplantation and death in mice inoculated with the same transplanted tumor growing in the same host at different locations.* — The tumor used for this experiment was a Z (C_3H) mammary adenocarcinoma which arose spontaneously in a Z (C_3H) breeder mouse and has been maintained in this laboratory for 47 successive passages

into ZBC mice. This tumor was transplanted in two different locations in the same mouse. At a given time, and when one of the tumors reached a mean size of 1.40 cm and the other, of 1.02 cm, both tumors were excised and a tumor cell suspension prepared from each one and injected at the same concentration into two groups of young male ZBC mice. In both groups of inoculated mice the "take time" and the "death time" were determined. The results (Table II) showed for the small tumor a take time of 22 days and a death time of 52 days. However, the take

TABLE II

Take time and death time of mice inoculated with the same tumor of different size, growing in the same host at different locations

Tumor	Size (cm)	Tumor cell concentration %	Nº of mice with tumors	Take time (days)	Death time (days)
Z transplanted	1.02	5.0	9/16	22	52
Z transplanted	1.40	5.0	16/16	15	38

time for the large tumor was 15 days and the death time, 38 days. The number of mice with tumors obtained in both groups was also different, for, whereas all inoculated mice with the large tumor developed tumors, 7 out of 16 mice inoculated with the small failed to produce tumors.

C. *Time of transplantation and death in mice inoculated with a given tumor at different size.* — The results of the experiments described led us to investigate whether the same tumor would have different transplantability and pathogenicity when inoculated at different sizes. This was accomplished by taking successive samples fron the same tumor at different sizes and transplanting them into normal mice at the same concentration. Biopsies of the tumor were made, removing a piece of it and preparing a tumor cell suspension which was inoculated into normal homologous mice. Two to three samples from the same tumor were tested.

Table III-A shows the results obtained when a Z spontaneous tumor was used. When the tumor attained a mean diameter of 1.00 cm, a biopsy of the tumor was made and a tumor cell suspension prepared at a 5 % concentration in saline. Such a suspension, injected subcutaneously in an amount of 0.25 cc into 8 normal ZBC female mice, did not produce tumors. A second biopsy from the tumor when it reached a size of 1.60 cm, injected at the same concentration into another group of 8 mice, produced tumors in 5 animals. The take time was 51 days and the death time, 134 days. Finally, a third sample was taken when

TABLE III

Effect of tumor size on the take time and death time after transplantation

Series	Experiment	Tumor	Size cm (Mean)	Tumor cell concentration %	Nº of mice with tumors	Take time (days)	Death time (days)
A	124 C	Z spontaneous	1.0	5.0	0/8	—	—
	124 C	Z spontaneous	1.6	5.0	5/8	51	134
	124 C	Z spontaneous	2.6	5.0	8/8	24	92
B	124 E	Z transplanted	1.1	8.0	6/6	39	90
	124 E	Z transplanted	2.4	8.0	7/7	24	74
	124 E	Z transplanted	3.0	8.0	12/12	19	56
C	124 H	A spontaneous	1.1	5.0	4/8	41	80
	124 H	A spontaneous	1.8	5.0	6/8	26	68
D	124 G	A transplanted	1.0	5.0	4/8	52	102
	124 G	A transplanted	2.6	5.0	8/9	16	60

the tumor was 2.60 cm in diameter, which, when inoculated at the same concentration into a third group of mice, produced tumors in all the animals with a take time of 24 days and a death times of 92 days.

Table III-B shows the results obtained when a transplanted Z tumor was used. This tumor was used in this experiment after 4 successive passages into ZBC mice. The first sample, taken when the tumor had an average size of 1.10 cm and inoculated at an 8 % concentration into a group of normal ZBC female mice, produced tumors in all mice inoculated, with a take time of 39 days and a death time of 90 days. The second sample, taken when the donor tumor reached a diameter of 2.40 cm and inoculated at the same concentration into a group of normal mice, produced tumors in all the animals, with a take time of 24 days and a death time of 74 days. Finally, a third sample was taken when the donor tumor was 3.00 cm in diameter. The inoculation of this sample at the same concentration produced tumors in all injected mice with a take time of 19 days and a death time of 56 days.

Table III-C shows the results obtained when an A spontaneous mammary tumor was used. Biopsies of the tumor taken when it was 1.10 cm and inoculated at a 5 % concentration into 8 normal ABC mice, produced tumors in four, the take time being 41 days and the death time, 80 days. The second sample, taken when the donor tumor was 1.50 cm in diameter and inoculated at the same concentration, produced tumors in 6 out of 8 mice, the take time being 26 days and the death time, 68 days.

Table III-D shows the results obtained when an A transplanted tumor was used. That particular tumor was used in this experiment after being transferred 2 successive times into ABC mice. A sample, excised when the tumor reached a size of 1.00 cm and inoculated into normal ABC female mice as a 5 % concentration, produced tumors in 4 out of 8 mice (take time, 52 days; death time, 102 days).

The second sample, taken from the tumor when it had attained 2.60 cm in size and inoculated at the same concentration into 9 normal female mice of the ABC stock, produced tumors in 8, the take time being 16 days and the death time, 60 days.

D. *Take time and death time determination by using a transplanted tumor at different sizes and inoculated at various concentrations.* — In these experiments, the same tumor as in experiment B was used. When that tumor attained a size of 0.9 cm, a biopsy was made and a tumor cell suspension in saline prepared at 5, 2.5 and 1 % concentrations. Three groups of female mice of the ZBC stock were used as recipient hosts. One group was inoculated subcutaneously with 0.25 cc of the tumor suspension prepared at 5 % concentration; a second group received the same amount of tumor suspension at 2.5 % concentration, and a third group was inoculated with the same amount of the tumor cell suspension prepared at a 1 % concentration.

The results (Table IV) showed that the take time in the three groups of inoculated mice was the same (28 days) while the death time was 57, 58 and 55 days, respectively.

When the donor tumor reached a size of 1.60 cm, a piece of the tumor was excised and a tumor cell suspension prepared in the same

fashion. Three groups of ZBC mice were then inoculated with the three different concentrations. The results showed that the take time for each group was 10, 14 and 14 days, respectively and the death time, 39, 40 and 37 days, respectively.

Finally, when the donor tumor attained a size of 2.40 cm, a final portion of the tumor was removed and a tumor cell suspension prepared in the same way as before. Again, three groups of ZBC female mice were then inoculated with the three different concentrations. It was

TABLE IV

Effect of tumor size on the take time and death time (Z tumor after 74 passages) using different tumor cell concentrations

Size of donor tumor (cm)	Tumor cell concentration %	Nº of mice with tumors	Take time (days)	Death time (days)
0.9	5.0	4/6	28	57
	2.5	4/7	28	58
	1.0	4/8	28	55
1.6	5.0	7/7	10	39
	2.5	8/8	14	40
	1.0	8/8	14	37
2.4	5.0	10/10	8	37
	2.5	10/10	12	40
	1.0	10/10	12	40

found that the take time for the three groups was 8, 12 and 12 days, respectively, the death time being 37, 40 and 40 days, respectively.

It can be noticed in this experiment that when the donor tumor was 0.9 cm, roughly half of the inoculated mice grew the tumor, irrespective of the concentration used, while all of the mice grew the tumor when the donor tumor was 1.60 and 2.40 cm in diameter, respectively.

E. *Intravenous inoculation of a given tumor at different size.* — In these experiments the production of lung tumorous nodules after the intravenous injection of a given tumor at different size has been studied. The tumor used was the same as in experiments B and D. The experiment was done by taking different samples from the same tumor at different sizes and injecting them as a 0.5 % concentration in saline, into the tail vein of normal mice of the ZBC stock. The total volume injected was 0.2 cc. At 7, 10 and 15 days later, mice of different groups

TABLE V

Production of lung tumor nodules after the intravenous inoculation of the same tumor at different sizes

		Number of mice with tumorous lung nodules			
Size of donor tumor (cm)	Tumor cell concentration %	7 days after inoculation	10 days after inoculation	15 days after inoculation	Total
0.80	0.5	0/6	0/6	0/6	0/18
1.50	0.5	2/6	6/6	6/6	14/18
1.85	0.5	4/6	6/6	6/6	16/18

were sacrificed, the lungs dissected out and the presence of tumorous nodules on the surface of the lungs determined under a dissecting scope.

The results (Table V) showed that the intravenous injection of the sample taken when the tumor was 0.8 cm did not produce lung nodules in the recipient mice killed at 7, 10 and 15 days. A new sample taken when the tumor reached 1.50 cm in size and injected at the same concentration as before produced nodules in 2 out of 6 mice of the group sacrificed at 7 days, and in all mice sacrificed at 10 and at 15 days after the inoculation. Finally, the sample taken when the tumor was 1.85 cm in size and injected at the same concentration as before, produced lung nodules in 4 out of 6 animals in the group sacrificed at 7 days and in all mice killed at 10 and 15 days after the inoculation.

Discussion

The results of the experiments described suggest that the transplantability and pathogenicity properties of mouse mammary tumors might be different, depending upon the size of the tumor at the time of its transplantation. In fact, it was shown that two spontaneous tumors of different sizes, ocurring in the same mouse and transplanted into two grops of normal susceptible mice, had different take times, the larger one having the shorter take time. However, if from each of the two groups of tumorous mice. two tumors were selected having the same size, and retransplanted into two groups of normal mice, then the same take times were observed (Table I, A and B). This is also true when the times of transplantation and death were determined in mice inoculated with two different size tumors taken from a mouse bearing the same tumor at different locations (Table II).

Differences in the take time and in the death time of the hosts were observed when samples of the same tumor were transplanted at different sizes, keeping the tumor cell concentration and the size of the inoculum constant. Here again, small size tumors had a longer take time than the same tumor transplanted at a larger size. Also, mice inoculated when the donor tumor was small, lived longer than mice inoculated with the same tumor when larger.

The number of mice with tumors obtained after the inoculation at a given concentration may be different, depending also on the size of the

TABLE VI

Effect of hypophysectomy or adrenalectomy on the growth of spontaneous mammary tumors of differente sizes

Animals showing:	Nº of mice	Tumor size before the operation (cm) (mean)
Complete regression of the tumor	6	0.72
Temporary regression of the tumor	11	1.06
No effect on the tumor growth	69	1.20

donor tumor at the time of transplantation. This is clearly seen in experiments on Tables 1-A, 2, 3 and 4.

As it would be expected, the intravenous injection of samples of the same tumor taken at different sizes and injected at the same concentration also gave similar results in the development of lung tumors.

These results are in agreement with those reported by Browning (1948) suggesting that "autonomy" is not present in small tumors, but it appeared gradually with the increase in age or size of the tumor. This, according to our experience, is also true for transplanted tumors, even for those used after 47 successive passages, which would indicate that tumors that have their "autonomy" fully developed because of successive passages may still show different degrees of transplantability, depending on the size or age of the tumor when transplanted.

It is rather difficult at this time to give an explanation of the results obtained. It is well known that the take time and the number of takes of a tumor when transplanted might depend, to a certain extent, upon the size of the inoculum in terms of actual number of tumor cells

inoculated. On that basis, it would be necessary to assume that the number of malignant cells per unit of tumor tissue mass should be less in small tumors as compared with large ones. That this might not be the case was shown by Zahl and Drasher (1947) who, working with a transplantable sarcoma, have reported that the percentage of viable tumor cells was larger in young than in old tumors.

The fact that Browning has shown differences in the transplantability properties of spontaneous mammary tumors between small and large ones after transplanting them by the trocar technic, that is to say by introducing an almost equal size bit of tumor into the anterior chamber of the eye, perhaps is an indication that the phenomenon observed cannot be explained on cell concentration only, since by introducing a bit of tumor, the concentration of tumor cells must be well above any critical value.

Whatever the real explanation of the phenomenon might be, it seems clear that the transplantability and lethality of a given mammary tumor, spontaneous as well as transplanted, is different, depending on the size or age of the tumor when transplanted.

Since the changes in these properties of tumors may take place while growing in the same host, it would be likely to assume that some other properties would also change. In this respect, it is interesting to point out at this time that perhaps the response of a given tumor to a pharmacological or therapeutic agent, might also be different depending on the size or age of the tumor at the time of treatment. In fact, one of us (C. M.), by doing either hypophysectomy or adrenalectomy in mice bearing spontaneous tumors of different sizes, has shown that those operations only produced complete tumor regression when done in mice bearing fairly small tumors, but it did not affect the growth of the larger ones (see Table VI).

SUMMARY

Spontaneous, as well as transplanted mammary tumors inoculated either subcutaneously or intravenously, have shown differences in transplantability and lethality, depending upon the size of the tumor at the time of its transplantation, the small tumors showing a longer take time and death time than the larger.

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EFFECTS OF ANOXIA ON THE PERFUSED CAT LIVER

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IN A PREVIOUS communication (1) it was reported that cat livers perfused with heterologous blood were normal in histological appearance and capable of glycogen synthesis, of transformation of fructose into glucose, of bromosulphonphenolphthalein (BSP) fixation and of production of bile.

This preparation was developed fundamentally for testing the effects of pharmacological agents on a functionally depressed liver. In view of this and since cellular anoxia probably plays an important role in liver disease it was considered expedient to study the effects of anoxia on the functions listed. The results of this study are reported.

METHODS

Livers from adult cats of both sexes were used. Two types of experiments were carried out, which in the future will be referred to as experiments of type A and of type B. Livers in type A experiments were perfused as previously described (1) using the system shown in figure 1, with the perfusion fluid in equilibrium with 100 per cent oxygen. In these conditions, the concentration of oxygen in the perfusion medium was about 4 volumes per cent. In experiments of type B, the closed system shown in figure 2 was used. Oxygen was taken intermittently from a reservoir in equilibrium with atmospheric pressure through a water seal. The carbon dioxide liberated was absorbed in a vessel containing filter paper saturated with a concentrated solution of potasium hydroxide which communicated with the oxygenation chamber. At equilibrium this chamber contained a constant mixture of oxygen and carbon dioxide which mantained a concentration of approximately 0.5 volumes oxygen percent in the perfusion fluid. In both types of experiments, defibrinated horse blood diluted with balanced salt solution (2) to a hematocrit of 20 percent was used. The flow in both types of experiments was constant at about 100 milliliters per minute.

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In each experiment the increase in liver weight, the production of bile, and the glucose concentrations in the course of the perfusion were recorded. In some cases, sections of the liver at the end of the experiment were fixed and stained. In several cases, the rate of disappearance of fructose and its transformation into glucose, as well as the rate of BSP fixation were measured. The methods of analysis for these substances and for oxygen concentration in the perfusion fluid were those in common use ([3], [4], [5], [6]).

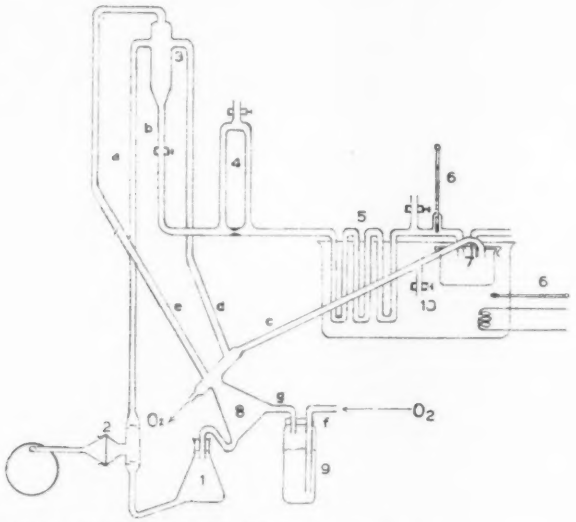

FIG. 1. — *Perfusion apparatus. Open system.*
(1) *Main reservoir;* (2) *rubber and glass pump;* (3) *reservoir for controlling perfusion pressure;* (4) *differential pressure flowmeter;* (5) *heating coil;* (6) *thermometer;* (7) *container for liver;* (8) *film oxygenator;* (9) *oxygen humidifier;* (10) *water bath;* (a) *blood from pump to reservoir;* (b) *to the liver;* (c) *from liver to oxygenator;* (d) *shunt for excess output from pump;* (e) *air connection for balancing pressures;* (f) *oxygen inlet;* (g) *entrance to the oxygenator;* (h) *cannula in bile duct.*

RESULTS

A. Histological changes. — The histological appearance of livers from both types of experiments was essentially similar. However, a slightly greater perilobular congestion and extravasation in portal spaces could be observed in sections from poorly oxygenated organs [1]. The increase in weight due to said congestion and extravasation in anoxic livers was significantly greater than that in the well oxygenated ones. Average weight increase in 37 experiments of type B, expressed as percentage of the initial weight, was 34.0 ± 22.2, while the average increase in 31 experiments of type A was 23.2 ± 10.2. The "t" test applied to the difference in means gave a value of 2.56, corresponding to a value of "P" between 0.01 and 0.02.

B. Production of bile. — No difference in the amount of bile produced by either type of experiment was observed. The average production in milliliters per hour in 17 experiments of type B was 0.69 ± 0.44. This is similar to the average production, 0.73 ± 0.25, already reported (1) for experiments with maximally oxygenated perfusion fluid.

C. Glucose equilibrium. — When the concentration of glucose was measured in the course of type B experiments in which no glucose had

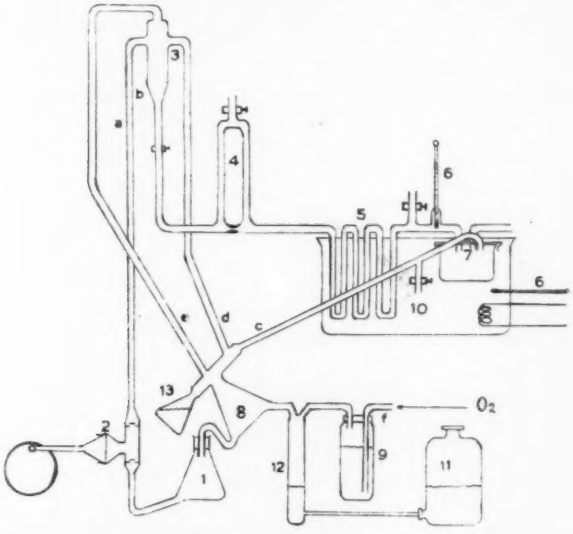

FIG. 2. — *Perfusion apparatus. Closed system.*
(1) *Main reservoir;* (2) *rubber and glass pump;* (3) *reservoir for controlling perfusion pressure;* (4) *differential pressure flowmeter;* (5) *heating coil;* (6) *thermometer;* (7) *container for liver;* (8) *film oxygenator;* (9) *oxygen humidifier;* (10) *water bath;* (11) *calibrated oxygen reservoir;* (12) *water tank for balancing with atmospheric pressure;* (13) *carbon dioxide absorption chamber;* (a) *blood from pump to reservoir;* (b) *to the liver;* (c) *from liver to oxigenator;* (d) *shunt for excess output from pump;* (e) *air connection for balancing pressures;* (f) *oxygen inlet;* (g) *entrance to the oxygenator;* (h) *cannula in bile duct.*

been added, equilibrium similar to that of type A experiments was observed. The mean equilibrium concentration in experiments of three hours duration was 2.8 ± 1.9 mg. of glucose per milliliter.

When large amounts of glucose were added to the system, a difference was observed between the anoxic and the well oxygenated livers. In the latter a steadily diminishing glucose concentration in the perfusion fluid could be shown, whereas in the anoxic livers the glucose level remained constant or even increased. This happened in 46 out of 54 type B experiments. The remaining 8 cases along with 26 experiments with full oxygenation of the fluid showed a decrease in the glucose concentration. In the latter the average decrease was 59.3 ± 23.0 expressed

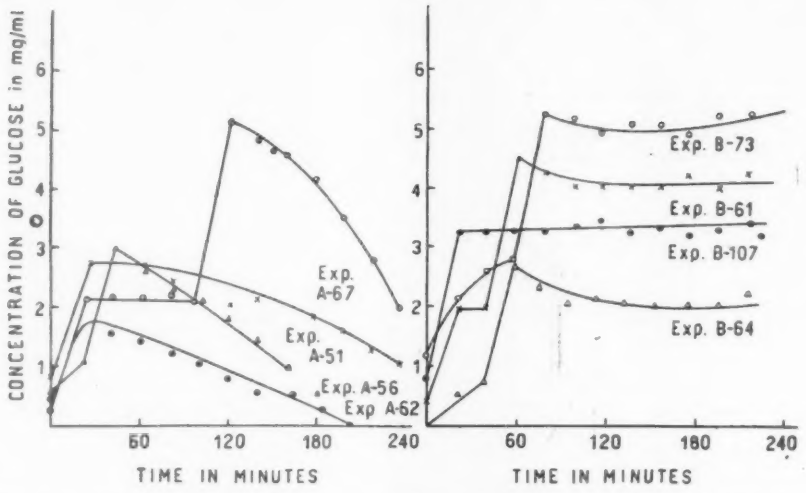

FIG. 3. — *Comparison between the rates of disappearance of fructose in either type of experiment. Abscissae represent time after the addition of 1 gram of fructose into the perfusion fluid. In each curve the first point represents the calculated theoretical values.*

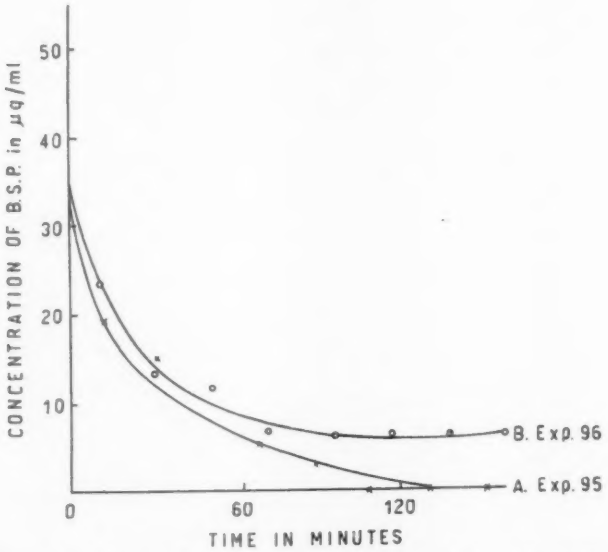

FIG. 4. — *Reducing substances in experiments of type A and type B to which fructose was added. In each case F indicates the addition of 2 g of fructose into the system. The first point in the fructose curves represents the calculated maximum value.*

as percent decrease from the value observed after the addition of the glucose. That is, the final glucose level was about 60 percent less than the maximum initial level. On the other hand, in the 54 anoxic livers, the final level was higher than the maximum initial level, on an average of 1.8 ± 29.1, also in percent over the maximum initial level. Figure 3 shows representative graphs for experiments of either type.

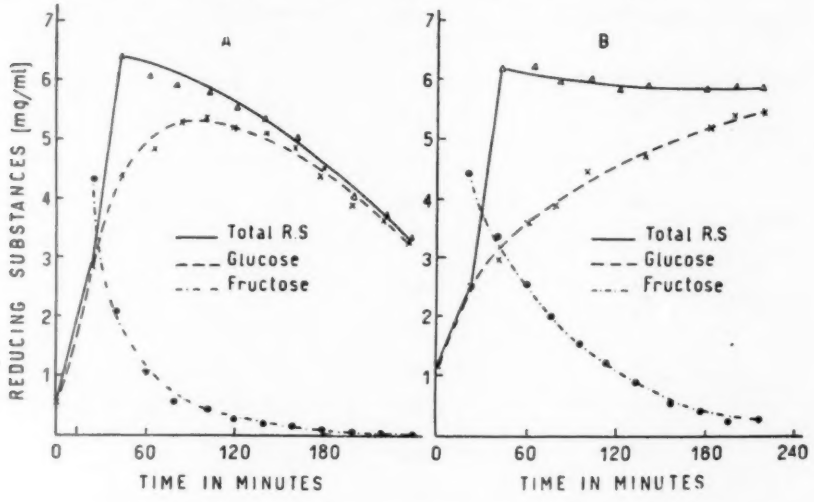

FIG. 5. — *Comparison between the rate of disappearance of BSP in an experiment of type A and one of type B. The abscissae represent time after the additon of 25 mg of BSP into the system. In each curve the first point represents the calculated maximum value.*

D. Disappearance of fructose. — Addition of fructose to the perfusion fluid in both types of experiments resulted in its disappearance at a similar rate (fig. 4). However, it was observed that the maximum fructose concentration after addition of the substance was higher in type B experiments, indicating less rapid initial transformation and that in most cases when no fructose was circulating in type A experiments a small amount was detectable in the perfusion fluid of experiments of type B. In table I initial and final fructose levels for pairs of experiments in which the rate of removal of fructose were compared are shown.

It was interesting to compare the levels of glucose and of total reducing substances in either type of experiment when fructose was added to the system (fig. 5). The transformation of fructose into glucose is easily seen in both cases, as well as the fact that in type A experiments the glucose tends to disappear at the end of the experiment whereas in type B experiments, although fructose is converted into glucose, the latter remains circulating throughout the period of observation.

TABLE I

Disappearance of fructose in pairs of type A and type B experiments carried out on the same day

Date	Experiment Nº	Type	Time Minutes	Theoretical level µg/ml	Initial level µg/ml	Final level µg/ml
1-13-54	80	A	100	2.2	1.00	0.00
	81	B	100	2.2	1.60	0.40
1-15-54	85	A	240	2.2	1.60	0.00
	86	B	240	2.2	1.75	0.15
1-20-54	95	A	220	2.2	1.70	0.00
	96	B	220	2.2	1.85	0.15
1-21-54	99	A	200	2.2	1.60	0.25
	100	B	200	2.2	1.80	0.70
1-26-54	108	A	220	4.4	2.1	0.10
	109	B	220	4.4	3.2	0.20

TABLE II

BSP levels in the perfusion fluids of two pairs of type A and type B experiments

Date	Experiment Nº	Type	Time Minutes	Theoretical level µg/ml	Initial level µg/ml	Final level µg/ml
1-20-54	95	A	130	55	19.4	0.00
	96	B	130	55	23.0	0.45
1-21-54	99	A	70	55	18.0	0.00
	100	B	70	55	26.8	0.40

E. BSP fixation. — BSP added to the system disappears rapidly from the perfusion fluid in both types of experiments. Figure 6 represents typical curves from each type, and Table II shows the data from two paired experiments. It can be seen that, though desappearance is rapid in both cases, the first determination was slightly higher for type B experiments, and some BSP remained in circulation in type B experiments at a moment when in type A experiments the substances had disappeared completely.

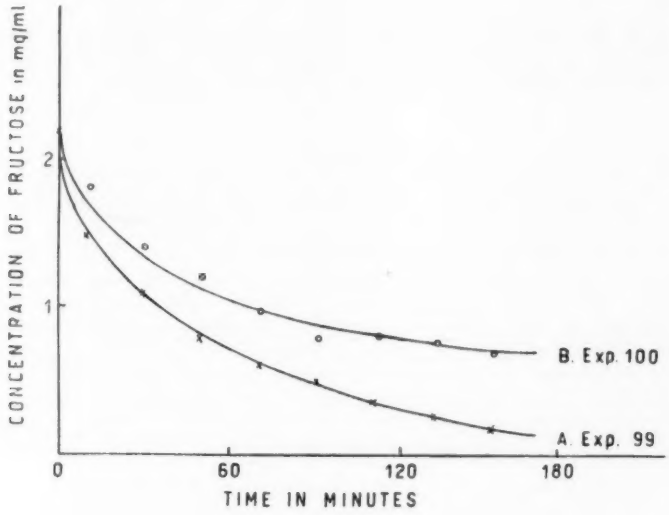

FIG. 6

DISCUSSION

It is apparent that most of the functions studied are carried out at approximately similar rates in livers perfused with fully oxygenated fluids and in those perfused with an anoxic medium. Thus, bile production was almost equal in both types of experiment, and the transformation of fructose into glucose and BSP fixation were only slightly more efficient with full oxygenation.

Although the greater increase in weight and the portal congestion found in type B livers might imply a functional impairement that would render the organ inadequate for other studies, it is probable that the almost complete normality of the functions refered to abové constitutes sound evidence of the viability of the preparation.

On the other hand, a clear difference between experiments of either type was observed in what refers to the handling of carbohydrates. The decreasing glucose level in fully oxygenated experiments seems to indicate its conversion into glycogen whereas the steady or increasing glu-

cose levels in type B experiments points towards an equilibrium in which glycogenolysis is the dominating factor. These observations may be interpreted as indicating that functions requiring little expenditure of energy (BSP fixation-transformation of fructose into glucose) are but slightly influenced by the amount of oxygen available, whereas a function such as glycogenesis which implies high energy utilization is more clearly dependent on the degree of oxygenation. The capacity of the liver for production of bile probably has more numerous and more complex limitations in a perfused liver, so that the influence of anoxia may not be evident.

It may be that the capacity of the liver for glycogen synthesis can become a useful criterion for studies with pharmacological agents capable of modifying the processes of energy utilization.

SUMMARY

The influence of anoxia on perfused cat livers was studied in regards to bile production, bromosulphonphenolphtalein fixation, transformation of fructose into glucose and glycogen synthesis from glucose. It was found that the first function referred to above was not modified when anoxic perfusion fluid was used and the following two were only slightly altered, whereas the capacity for glycogen synthesis was clearly depressed. The possibility of using the capacity of the liver to form glycogen as a criterion of function in the pharmacological evaluation of substances acting on the processes of energy utilization is suggested.

We wish to express our gratitude to Dr. Edmundo Rojas, of the *Hospital de Enfermedades de la Nutrición*, who carried out the histologic studies.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

Buenos Aires, June 2nd, 1955

Local effect of hydrocortisone in hypertrophic scars. BY R. E. MANCINI AND S. STRINGA. *(National Institute of Endocrinology, Godoy Cruz 1221, Buenos Aires).*

The action of alloxan in the turtle *Pseudemys D'Orbignyi D and B.* BY NABUCO LOPES. *(Institute of Experimental Physiology. Faculty of Medicine, Porto Alegre, Brazil).*

Fixation of calcium 45 in bones and teeth of rats under experimental fluorosis. BY V. H. CICARDO, J. C. MURACCIOLE AND F. J. DE LERNER. *(Institute of Biological Physics, Faculty of Medicine, Buenos Aires).*

Relation of crystal size to estrogenic activity of parahydroxypropiophenone. BY V. G. FOGLIA, J. C. PENHOS AND E. MONTUORI. *(Institute of Biology and Experimental Medicine, Buenos Aires).*

The administration of parahydroxypropiophenone original powder to rats in a high dose sustained for a long time, orally or by subcutaneous injection (in saline or corn- oil) did not provoke estrus nor inhibit the pituitary.

The same powder acquired estrogenic activity when injected in sesame oil or transformed into microcrystals of 2.000 μ^3 or less.

The microcrystals were active orally or injected and produced all the typical phenomena of estrogenic substances.

Active microcrystals of 2.000 μ^3 or less when transformed into larger ones (3.000 to 10.000 μ^3) of the same shape became inactive. If these large microcrystals are retransformed into small ones they recover their estrogenic activity.

All these results indicate that the activity of PHP is dependent on the size of its crystals.

In conclusion, PHP appears to be a weak estrogenic substance, the action af which depends probably upon the rate of absorption by the animal.

Changes in human electrodermograms, sweat secretion and skin temperature, produced by chloropromazine (4560 RP). BY N. CLERC, M. TURNER AND E. L. BÉRARD. *(Juncal 1695, Buenos Aires).*

The authors made several comparative tests of sweat secretion, skin temperature and electrodermogram variations before and after the slow intravenous injection of chloropromazine (4560 RP).

Sweat secretion was artificially stimulated by intradermal injection of 1/1000 sol of Adrenaline and by doses of 0.02 mg of carbaminoilcholine (Doryl), and was registered with filter papers impregnated with an adhering powder containing Bro-

mophenol Blue. *Skin temperature* was measured with the MacKesson Dermalor. The *electrodermogram* was obtained with two electrodes placed on the skin of the hand and connected to the amplifier circuit of a Kaiser's electroencephalograph.

After the injection of 4560 RP, the sweat response to adrenalin was negative, while the contralateral nocireptive stimulus response was positive. The electrodermogram response was inhibited as an effect of the injection of the chloropromazine.

The proximal skin temperature (arms and cheeks) diminished in every case, while the distal skin temperature (hands and legs) increased.

Action of growth hormone on the blood pressure of rats with reduced renal mass. BY E. BRAUN-MENÉNDEZ AND J. C. PENHOS. *(Institute of Biology and Experimental Medicine, Buenos Aires).*

Somatotrophin (1) (2 mg per rat per day) was administered subcutaneously during variable periods of time (8-30 days) to 10 rats with reduced renal mass (ligature of 8 in the left kidney and right nephrectomy). We selected for this experiment animals which, in spite of the reduced renal mass, had maintained a blood pressure within normal limits during 2 to 6 months after the operations. (Only one had a moderate hypertension). In 9 of the 10 rats a definite rise in blood pressure to hypertensive levels was observed following the administration of somatotrophin.

As the diet commonly used by us is rather poor in protein (16 % of dry residue) some animals were fed a richer protein diet (32 %). In others 1 mg of folic acid was given together with somatotrophin. As the number of rats used is small no definite evidence could be obtained of the potentiating action of either folic acid or a rich protein diet; but it can be affirmed that somatotrophin caused hypertension even with diets poor in protein and without the addition of salt.

In rats with reduced renal mass previously rendered hypothyroid by I^{131}, somatotrophin was ineffective; the addition of small doses of thyroid powder was necessary in order to evidenciate the hypertension producing action of somatotrophin.

These experiments substantiate the hypothesis that hypertension appears whenever substances with renotrophic action are given to rats with reduced renal mass, i. e. unable to respond to the growth stimulus with an increase in kidney mass and function.

(1) STH (Beef growth hormone) 521-A from Armour Research Laboratories.
This papel will appear "in extenso" in the *Revista de la Sociedad Argentina de Biología.*

Spermiation in *Bufo arenarum* by action of oxalates. BY J. C. PENHOS AND A. F. CARDEZA. *(Institute of Biology and Experimental Medicine, Costa Rica 4185, Buenos Aires).*

Sodium oxalate (40-50 mg) can produce spermiation in *Bufo arenarum* (in 5/20 toads after 50 mg, by subcutaneous injection). The Sertoli cells are vacuolated or its apical extremity desintegrated, the spermatozoa are liberated and become free in the fluid of the dilated seminiferous tubes. In hypophysectomized toads (without *pars distalis*) the spermatozoa are found exceptionally in the urine (1/20 after 50 mg), but microscopical sections showed spermiation (liberation of spermatozoids). Sodium oxalate produces also liberation of spermatozoa *in vitro* in small discs of testicles.

This papel will appear "in extenso" in the *Revista de la Sociedad Argentina de Biología.*

Pancreatic diabetes in normal and hypophysectomized turtles. BY V. G. FOGLIA, E. M. WAGNER, M. DE BARROS AND M. MARQUES. *(Institute of Physiology, Faculty of Medicine, University of Porto Alegre, Brazil).*

Total pancreatectomy produces rapid and intense increase of blood sugar level in the turtles *Chrysemis d'Orbignyi* and *Phrynops hilarii*. The technic of pancrea-

tectomy is more simple in *Phrynops* and the survival is longer (more than 1 month). The blood sugar level raises 3-5 times upon the normal level, after 48 hours it remains stable and never falls again.

Hypophysectomy lowered the glycemia (38 mg/100 ml, average of 12 hypophysectomized *Chrysemis*, 76 mg/100 ml average of 12 normals). Pancreatectomy produces less increase of blood sugar in hypophysectomized turtles. This attenuation of diabetes is permanent in *Chrysemis* and disappears after 3-4 days in *Phrynops*.

Glycogen variations are not constant. In hypophysectomized turtles, glycogen decreases in liver and muscle. In pancreatectomized turtles, it decreases in liver, less in muscle and increases in heart.

The diabetic high level of blood sugar is decreased by insulin until normal or subnormal levels are attained.

This papel will appear "in extenso" in the *Revista de la Sociedad Argentina de Biología*.

Buenos Aires, July 7th, 1955

Permeability to I^{131} of normal and fluorated teeth. By V. H. Cicardo and J. C. Muracciole. *(Center of Phthisiology).*

Protection of succinatedehydrogenase thiols by polysulfonated compounds. By A. O. M. Stoppani and J. A. Brignone. *(Institute of Biochemistry, Faculty of Medicine, Buenos Aires).*

Buenos Aires, August 4th, 1955

Sensitivity to histamine of rats with accesory or demedulated adrenals. By R. H. Houssay. *(Institute of Biology and Experimental Medicine, Buenos Aires).*

Adrenalectomized rats, in spite of the development of large accesory adrenals (35 to 40 mg), maintain a great sensitivity to histamine intoxication (intravenous or intraperitoneal injections).

After unilateral adrenalectomy the sensitivity is not changed. The average gland weight is 22 mg.

After removal of the adrenal medulla, the sensitivity to the toxic action of histamine is intermediate between the sensitivity of normals and adrenalectomized rats. Average weight of glands is 36 mg.

Cortisone and adrenalin have a protective action; the first is most active.

Adrenocorticotrophin (ACTH) protects the normal rat, slightly the rat without adrenal medulla and has no protective action on the adrenalectomized rat that has developed accesory glands. ACTH increases definitely the weight of the adrenals of normal rats, slightly or not the weight of the adrenal deprived of medulla and has no action on the weight of the accesory glands. ACTH produces eosinopenia in the normal rat, has slight or no action in rats without adrenal medulla and fails to produce eosinopenia in rats with accesory adrenals.

Accesory adrenals are practically insensitive to the action of endogenous or exogenous ACTH. That seems to be the principal reason of the sensitivity to histamine.

The sensitivity is due mainly to the lack of cortical function. Adrenal medulla has an accesory protective action.

Cancerous transformation of the ovary graft in the kidney of castrated rats. BY E. FELS. *(Maternity Institute. Ministery of Public Health, Buenos Aires).*

The ovarian graft in the kidney may change into a functional tumor although with less frecuency than the graft in the liver or spleen. This fact confirms the opinion that the inactivation of the estrogens by the liver cannot be the reason of the tumo formation.

Pressor responses of the cerebral cortex in sympathectomized dogs. BY V. H. CICARDO AND E. BREITER. *(Institute of Biological Physics. Faculty of Medicine, Buenos Aires).*

In the dog, bilateral sympathectomy between 5 D and 12 D diminishes the rise in blood pressure brought about by stimulation of the motor and premotor areas of the brain cortex.

Total adrenalectomy in sympathectomized dogs diminishes even more the blood pressure responses to cortical stimulation.

Protection by phosphate ions of succinatedehydrogenase thiol groups. BY A. O. M. STOPPANI AND J. A. BRIGNONE. *(Institute of Biochemistry. Faculty of Medicine, Buenos Aires).*

The inhibition of succinatedehydrogenase thiol groups by arsenicals and iodosobenzoate decreases by the presence of phosphate ions which effect cannot be replaced by similar concentrations of sulphate.

The protection of succinatedehydrogenase by phosphate is not necessarily bound to succinoxidase activation as other similar activators, like versenate, lysine and histidine do not protect succinatedehydrogenase.

Fluoride potentiates succinatedehydrogenase protection by phosphate in agreement with the hypothesis of the scarcely dissociated fluoride-phosphate-dehydrogenase complex.

Relationship between doses of thyrotrophin and thyroid cytologic coefficient of the guinea pig; statistic evaluation of the ecuation of the variables. BY E. DEL CONTE AND N. P. MORUZZI. *(Laboratorios Experimentales - Lavalle 332, Buenos Aires).*

Buenos Aires, September 1st, 1955

Action of thyrothophin on the blood pressure of rats with reduced renal mass. BY E. BRAUN-MENÉNDEZ AND J. C. PENHOS. *(Institute of Biology and Experimental Medicine, Costa Rica 4185, Buenos Aires).*

Thyroid stimulating hormone (TSH) of a potency of 3/10 USP units per mg was administered subcutaneously (2 mg per rat during 4 days and 4 mg for 2 more days) to 4 rats with reduced renal mass (ligature in 8 in the left kidney and right nephrectomy). Five rats were kept as controls. The blood pressure of these

9 animals had been taken weekly during 3 months following the kidney operations and was normal or moderately elevated. Administration of TSH caused a rapid and significant rise of the blood pressure to hypertensive levels. Two to three weeks after the end of the treatment the blood pressure returned to its original level. A second group of 10 rats with renal mass reduction of the same degree was injected with 800 μ C of I^{131}. Six of them received a maintaining dose of 2.5 μg of thyroxin per 100 g body weigth daily, the other 4 were kept as controls. A week after starting the thyroxin administration all the 10 rats were injected during 9 days with TSH (2 mg per rat per day). No rise in blood pressure was observed in these athyroid rats.

These experiments show that administration of thyrotrophin to rats with reduced renal mass causes an increase in blood pressure similar to that produced by the administration of high doses of thyroid powder or thyroxin. As thyrotrophin is ineffective in athyroid rats it can be assumed that its action is mediated through the secretion of endogenous thyroid hormone.

Action of gonadotrophins in the blood pressure of rats with reduced renal mass. By E. Braun-Menéndez, E. Krieger and J. C. Penhos. *(Institute of Biology and Experimental Medicine, Costa Rica 4185, Buenos Aires).*

Administration of gonadotrophin (10 U per day of a mixture of seric (PMS) and chorionic) during 20 or more days to normotensive or moderately hypertensive male rats with reduced renal mass (ligature in 8 of the left kidney and removal of the right kidney) caused a rapid rise in blood pressure to hypertensive levels. The injection of 10 U of seric (PMS) or chorionic gonadotrophin separately had a similar action. Smaller doses (5 and 2 U per day) caused also a rise in blood pressure. This effect was not observed in female rats.

The daily injection of 5 U of a mixture of both gonadothophins during 24 days did not modify significantly the kidney weight in male or female normal rats.

Prolonged administration of 5 U of a mixture of both gonadotrophins beginning 24 days before the renal mass reduction and continued for 2 more months aggravated considerably the course of the consecutive hypertension in male rats, which developed a severe hypertension with a high incidence of periarteritis nodosa. The same treatment had no effect in female rats.

Some aspects of an electromyographic study of the reflex activity in man. By J. Paillard (París) and M. Turner (Buenos Aires). *(Center of General Neurophysiology, College de France).*

A method of electric investigation of the reflexes in man is proposed, based in Hoffmann's technique and utilising rapid sequences of stimuli, of adequately selected intensities.

This method permits the dynamic exploration of the excitability state of the medullar centers in man and is particularly apt for the analysis of several central complex repercussions of which the authors give some examples.

Ovarian graft in the spleen of the male castrated rat. By E. Fels and E. G. Bur. *(Institute of Maternity, National Departament of Social Assistance, Buenos Aires).*

Ovarian grafts have been made in the spleen of 16 male castrated rats, obtaining between the 188 and 380 days, seven tumors that are identical to those appearing in females. There is no sign of androgenic action of these tumors.

It is known that the development of blastomas is related to the hypophyseal function. The identity of the tumors in both sexes suggests that no differences exist in the gonadotrophic function in castrated rats.

Modifications of the connective tissue in the albuginea of normal human testicle at different ages. By R. E. Mancini, F. Arrillaga O. Vilar and F. A. de la Balze. *(IIIrd Chair of Medicine, Faculty of Medicine, Charcas 2202, Buenos Aires).*

An histological and histochemical study was carried out on the modifications experimented by the connective tissue of the albugina of normal human testis during infancy, puberty, adulthood and senescence.

The following observations were made: 1º) from birth to the second infancy, the connective tissue of the albuginea (fig. 1, 2, 3, 4) progressively maturates from an embrionary type rich in juvenil fibroblasts, reticular fibers, mucopolysaccharides and vessels to an adult type characterized by adult fibroblasts, fibers and dense colagen bundles. This process is less intense in the deeper layers of the albuginea, in which embrionary connective tissue partially persists. 2º) in the puberty (fig. 5), and consequently with the beginning of the development of the germinal epithelium and Leydig cells, the albuginea experiments a marked growth at the expense of both the middle and deep layers. There is a reactivation of the connective tissue revealed by hyperplasia and hypertrophy of the juvenile fibroblasts and the fibers accompanied by an abundant vascularization. 3º) Later on, during the puberal maturation (fig. 6), the intensity of the cellular and fibrillar proliferation decreases in nearly all the layers, but persists moderately in the lower part of the middle layer and in the deeper layer. 4º) during the course of adult age (fig. 7) the albuginea thickened in the two outer layers, meanwhile the superficial layers developed a process of partial fibrohyalinosis. 5º) Finally, in senescence (fig. 8) the proliferative of the middle and deeper layers decreases and a tendency to the extension of the hyalinization from the superficial to the middle layer takes place. 6º) Histochemically, the juvenile fibroblasts were characterized by their content in cytoplasmatic ribonucleoproteins, which disappeared in the adult cells; the reactions for lipids, alkaline phosphatase and ascorbic acid were always negative in both cellular types. The techniques for the mucopolysaccharides were intensely positive in the embrionary connective tissue type and diminished with the fibrogenesis in the adult. Reactions for glycoproteins were moderately positive in the reticular fibers, the vessel's walls and juvenile colagen fibers.

Diabetogenic action of somatotrophin in the turtle Phrynops Hilarii. By M. Marques. *(Institute of Experimental Physiology, Faculty of Medicine, Porto Alegre, Brazil).*

Pituitary growth hormone, in the doses of 0.25 mg, 0.5 mg, 1 mg, and 5 mg per kg of body weight per day, produced transitory diabetes in the subtotal pancreatectomized turtle "Phrynops hilarii".

Subsequent treatmens diminishes the sensitivity to the hormone.

Normal turtles showed a marked sensitivity to the diabetogenic action of growth hormone.

During the period of hyperglycemia, lesions of the beta cells were observed: degranulation, vacuolization, pyknosis and necrosis. When the blood sugar fell to normal values, the aspect of the cells became normal.

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(3) Gómez, S. L., Pérez, J. M., López N. A.: *Acta physiol. Lat.-Amer.*, 1950, *1*, 43.

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milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Ångstrõm	Å	miligramo	mg	segundo	s
				milisegundo	ms

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